STUDY PROGRAM FOR

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BIOCHEMICAL MONITORING BY PAROTID SECRETION

FINAL REPORT

Beckman®

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FINAL REPORT

Space Engineering Department

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1.0 INTRODUCTION

This report by Beckman Instruments, Inc. is the result of a program on biochemical monitoring of biochemical changes in body fluids under varying conditions by parotid secretion.

In pursuit of this program more than two thousand analyses were made on body fluid samples from 102 different subjects. The subjects were of three distinct groups, viz., 1) normal, or general population; 2) hospitalized patients; 3) a select group of marine pilots. The homogenous third group was subjected to a series of body function tests. Sampling of this group for the various chemical components was also done during exercise and post-exercise periods. As a result, the present program offered a total of five test populations.

A significant addition was made to the original scope of this program through the efforts of the men in this specialized group. It was comprised of nine officers from the El Toro Marine Corps Air Station, California. They contributed a great amount of time, effort and body fluids and made possible the collection of some of the valuable data presented here. An expression of appreciation is extended to them for their participation in the program.

2.0 OBJECTIVE

In Phase I, the contractor undertook extensive laboratory experimental work and analysis to prove the feasibility of one or more physiochemical and biochemical methods for comparing the parameters listed in Table I. Work included extensive investigation and design of sampling and collection techniques.

TABLE I

Clinical Determinations

Test	No. Parotid Samples	No. Blood Samples	No. Urine Samples
1. pO_2	60	30	--
2. pCO_2	60	30	--
3. HCO_3	60	30	--
4. Urea	60	30	--
5. NH_3	60	30	--
6. Ca	60	30	60
7. Total Protein	60	30	30
8. Glucose	60	30	30
9. Cholinesterase	15	15	--
10. VMA	15	15	

3.0 SUMMARY

The feasibility of monitoring biochemical changes in parotid secretion under varying conditions has been demonstrated.

Simple body function tests rapidly elicited distinct changes in concentration of parotid components greatly in excess of those evidenced in other monitored body fluids. The potential use of parotid secretion as an indicator of physiological changes is clearly documented. A statistical comparison of mean concentrations of several components in parotid showed the marine control group to be separate from the normal and hospitalized populations. The ability to differentiate between groups with varying backgrounds is strong evidence that the parotid secretion is sensitive to physiological changes.

In general, material concentrations in parotid fluid are lower than in blood. However, reliable assays were routinely achieved for all the considered parameters except for cholinesterase and vanillylmandelic acid. It is believed that automatic measurements for partial pressures of oxygen and carbon dioxide and bicarbonate concentration can be evolved from present state-of-the-art techniques. Calcium, protein, and glucose require more effort, while urea and ammonia appear even more difficult with present day instrumentation.

Specific conclusions from the data obtained are:

- It is feasible to monitor biochemical changes in parotid secretion under varying conditions

- o Automatic measurements of several parameters are feasible
- o Non-stimulated flow rates are adequate for monitoring any one or two specific components. However, for sequential analysis of several components, stimulated flow rates are recommended.
- o Parotid fluid appears to be a mechanism to detect physiological changes; further work is necessary, however, to completely define these relationships.
- o Parotid fluid partial pressure of oxygen is a more pronounced indicator of metabolic changes than venous blood partial pressure of oxygen.
- o The effect of exercise on the level of calcium in parotid fluid is to elevate the concentration.
- o Ammonia concentration in parotid fluid reflects the effects of body function tests.
- o Cholinesterase and vanillylmandelic acid concentrations are below the levels of sensitivity of the methods currently available for their detection.
- o Parotid urea-nitrogen concentrations reflect the blood urea-nitrogen concentration and may be utilized to evaluate those metabolic conditions associated with variations in concentration of blood urea nitrogen.
- o Protein concentration can distinguish different populations; however, further study on protein concentrations in parotid fluid is necessary to establish the meaning of such variations in concentration.
- o The partial pressure of carbon dioxide in parotid fluid and the bicarbonate concentration of parotid fluid can distinguish between certain populations.

- Glucose concentration in parotid fluid can be utilized for studying the relationships of glucose metabolism under varying conditions.

4.0 RESULTS

The total number of analyses made for the various fluid components is shown in the sample status sheet on Page 4-2. Despite the fact that the sampling requirements have been greatly exceeded for most components, the data is minimal when divided into the various populations. It should be noted that the major portion of the data is based on stimulated parotid flow. This was necessary to obtain adequate volume since duplicate samples were analyzed in eight different procedures.

Stimulation caused a decrease in the concentration of the parotid components (except for post-exercise glucose), but did not otherwise change the relative levels of the components. Blood values were not significantly influenced. Non-stimulated flow data is included for comparison. The data presented is based on only a few cases.

The reduced data, permitting a comparison between the parotid, blood, and urine values, is presented in Appendices A, B, C, D, and E. Specific results comparing the test populations are shown below. These are for stimulated flow unless noted.

1) The mean values for stimulated parotid flow were similar for all test groups under quiescent conditions, viz., 1.14 to 1.2 ml/min when bilaterally collected.
2) With exercise, the control group stimulated parotid flow rate decreased to 0.84 ml/min, a drop of about 26%.
3) Non-stimulated flow rates under quiescent conditions averaged 0.04 ml/min.

NOTE: STRIPES INDICATE NUMBER OF DETERMINATIONS COMPLETED OVER THAT DESIGNATED BY PROPOSAL
NORMAL SUBJECTS
HOSPITALIZED SUBJECTS
CONTROL SUBJECTS
PAROTID Po2
PAROTID Pco2
PAROTID HCO3
PAROTID UREA-N
PAROTID NH3-N
PAROTID CALCIUM
PAROTID TOTAL PROTEIN
PAROTID GLUCOSE
URINE CALCIUM
BLOOD Po2
BLOOD Pco2
BLOOD HCO3
BLOOD UREA N
BLOOD NH3-N
BLOOD CALCIUM
BLOOD PROTEIN
BLOOD GLUCOSE
URINE PROTEIN
URINE GLUCOSE
PAROTID CHOLINE-TERASE
PAROTID VMA
BLOOD CHOLINESTERASE
BLOOD VMA
189
149
158
0
20
40
60
80
100
120
130

4) Average partial pressure of oxygen of parotid fluid was 95 mm Hg for the normal group, 87 for the hospitalized, and 81 for the control group.

5) The partial pressure of oxygen in parotid fluid decreased 31% after exercise. The blood value increased 9%.

6) Parotid carbon dioxide partial pressures varied from 32 mm Hg for the normal group to 34 for the hospitalized patients, and 38 for the control group.

7) The partial pressure of carbon dioxide was about 7% lower after exercise while the blood value dropped 10%.

8) Parotid bicarbonate values were 14.4 m moles/l for the normal group, 13.8 for the hospitalized, and became elevated to 21.6 for the control group.

9) After exercise, parotid bicarbonate had dropped 10% while the blood value decreased 16%.

10) Urea concentrations in parotid fluid were 9.2 mg% in the normal group, 12.1 in the hospitalized, and 8.8 in the control group.

11) Parotid urea increased 17.5% after exercise but blood decreased about 3.5%.

12) Parotid ammonia measured 127 ug% in the normal group, 142 in the hospitalized, and 114 in the control group.

13) Parotid ammonia increased rapidly during exercise, rising 48%. After exercise it dropped to an elevation of only 5%. Blood ammonia remained substantially constant with a post exercise drop of only 1%. Blood, of course, was not measured during an exercise period.

14) Mean calcium levels in parotid fluid for the various populations were 1.73 meq/1 for the hospitalized, 1.70 meq/1 for the normal, and 1.65 meq/1 for the control group.

15) Parotid calcium level increased 11.5% post exercise while blood increased 1.5%.

16) Protein concentrations in parotid fluid were 201 mg% for the normal group, 234 mg% for the hospitalized, and 131 mg% for the control marine population.

17) Post-exercise parotid protein increased 43% while blood went up 6%.

18) Glucose concentrations in parotid fluid are 0.95 mg% for the control group, 0.98 mg% for the hospitalized group, and 1.37 mg% for the control marine group.

19) Glucose, post-exercise, was up 177% while in blood it dropped about 7%. Non-stimulated flow also showed an increase, but only 35%, based on just 3 cases. Blood again decreased, 8% in this sampling.

It is evident that parotid secretion shows marked variation in different select groups and under varying metabolic conditions.

5.0 DISCUSSION OF DATA

Over 2000 analyses were made on parotid, blood, and urine samples on 102 different subjects from various groups of individuals. These groups are described, along with the sampling technique utilized, in Appendix F. The samples were analyzed by the procedures defined in Appendix G. The resulting data has been tabulated in Appendix A. An analysis of the data appears in Appendix C, D and E.

The five groups studies are defined as:

1) Normal: Individuals who are ambulant and not undergoing any known medical treatment.
2) Hospitalized: Individuals undergoing medical treatment within the confines of a hospital
3) Control: The pilots from the El Toro Marine Base prior to exercise
4) Exercise: The marines during the body function test period (30 minute biodynamic period)
5) Post-Exercise: The marines after the completion of the test period.

A discussion of the various components investigated is described in subsequent sections.

5.1 The Partial Pressure of Oxygen in Parotid Fluid

The partial pressure of oxygen in parotid fluid was determined by Beckman Model 160 Physiological Gas Analyzer. The instrument utilizes a polarographic technique for measuring pO_2. The stability, reproducibility and

sensitivity of the technique is described in Appendix G. Calibration of the instrument was achieved by using atmospheric oxygen partial pressure for the upper limit and a prepared bottled gas mixture of CO_2 - N_2 for zero oxygen partial pressure.

The pO_2 in parotid fluid ranged from a low of 30 mm Hg in the post-exercise marines to a high of 142 mm Hg in the normal population. The means for the five populations sampled ranged from a low of 55.6 mm Hg (of the post exercise marines) to a high of 95 mm Hg (for the normal group) as shown in Appendix A. By applying the t-test, an estimation of the probability that the means of these various samples are from different populations was tabulated in Appendix B. Those showing a highly significant probability of being different populations are:

1) Normal and exercise means
2) Normal and post-exercise means
3) Hospitalized and exercise means
4) Hospitalized and post-exercise means
5) Control and post-exercise means

A significant probability of being different populations is exhibited by:

1) Normal and control means
2) Control and exercise means
3) Exercise and post-exercise means

It would appear from the analysis that it may be possible to distinguish various populations by the partial pressure of oxygen in parotid fluid. Examination of the marine population which has been subjected to physical activity shows a significant decrease in the oxygen partial pressure in

the parotid fluid, viz., from a mean of 81.2 mm Hg to 66.6 mm Hg (Appendix A). This decrease in oxygen partial pressure continues and is still evident in the post-exercise mean. In fact, the post exercise mean is statistically significantly lower than the exercise mean (Appendix B).

It is possible to distinguish the three different populations (control, exercise, and post-exercise) by the oxygen partial pressure in parotid fluid (Appendix B). When comparing the venous partial pressure values for the pre- and post-exercise populations, it is not possible to distinguish between these groups. Thus, onemay speculate that parotid fluid is a more sensitive indicator than venous blood for evaluating the metabolic changes via the partial pressure of oxygen.

5.2 The Partial Pressure of Carbon Dioxide in Parotid Fluid

The partial pressure of carbon dioxide in parotid fluid has a lower mean value than the value of blood when measured by the Beckman Physiological Gas Analyzer with a Severinghaus-type electrode (Appendix A). The range measured was 17 mm Hg (from the post-exercise group) to 51 mm Hg (from the pre-exercise group). The means of the five groups ranged from a high of 37.8 mm Hg (marines) and a low of 31.8 mm Hg (the normal group). The t-test revealed that there was a 99% chance that the control group and the normal group are two different populations and that there was a 95% chance that the hospitalized and control groups are two different populations (Appendix B). When this control group is subjected to exercise, the mean values drop and approach the values obtained for the normal and hospitalized groups. From a statistical standpoint we can no longer distinguish between the groups.

Although it is not possible at this time to determine the significance of different populations for pCO_2 concentrations, it does appear that they do exist.

5.3 Bicarbonate Concentration in Parotid Fluid

Values for the bicarbonate concentration in parotid fluid were obtained employing the Beckman Physiological Gas Analyzer for measuring pH and pCO_2 of the fluid, and using these values in the Henderson-Hasselbalch equation (Appendix G). The range measured in parotid fluid was from a low of 5.7 millimoles per liter (normal group) to a high of 34 millimoles per liter (exercise group). The mean values of the five populations ranged from a low of 13.8 millimoles per liter (hospitalized group) to a high of 21.6 millimoles per liter (marine group). In the analysis of the normal and hospitalized groups it is not possible to distinguish any differences in population. They appear to come from the same population. However the marine control group appears to be a different population. The t-test shows a 0.999 probability of a different population (Appendix B). Exercise decreased the mean concentration by approximately 12%. There is a 0.9 probability that this exercised population is a different group from the control marines prior to the exercise cycle.

Why the mean concentration of bicarbonate in parotid fluid is so much higher in the marine population cannot be explained. However, this difference does appear to exist. The instrumentation was calibrated in an identical manner for each series of samples. The same calibration standards were used, i.e., buffer and prepared bottle gas. It is therefore highly unlikely

that the values obtained were due to an error in calibration standards or technique. It would appear then that bicarbonate concentration can be used to distinguish between certain populations.

5.4 Calcium Concentration in Parotid Fluid

Calcium levels in parotid fluid for the five test populations tend to have very similar mean values and standard deviations except for the post-exercise group which shows approximately 12% higher mean value (Appendix A).

This group can be recognized statistically from the other groups (Appendix B). The range found in parotid fluid for calcium is 1.0 meq/l to 3.6 meq/l. The control marine group had the lowest mean value of 1.65 mg% as compared to the highest mean value of 1.84 meq/l, which was the post-exercise marine group.

It would appear that the effect of exercise on the level of calcium in parotid fluid is to elevate the concentration. No such elevation can be seen in the blood mean values of these groups. Parotid fluid would thus appear to be a meaningful but yet uninterpreted indicator of change.

5.5 Ammonia Concentration in Parotid Fluid

The ammonia concentration in parotid fluid shows a significant increase when the control marine group is subjected to exercise. There is a mean value increase from 114 mg% to 179 mg%, a rise of 48%. After the completion of the exercise cycle the level dropped to the baseline values again (Appendix A). No significant difference in sample means can be found for the other groups. The range for ammonia concentration in parotid fluid is from a low of 45 mg% (marine control) to a high of 354 mg% (exercised marines). The mean levels of ammonia found in blood were approximately

10% higher for the groups measured. No information is available, however, for the exercising marine group for blood.

In general it would appear that ammonia concentration in parotid fluid reflects the effects of body function tests. Thechange in level is dramatic and appears to be consistent within the individual (Appendix D).

The probability of the control and exercise means which are measured from different populations is 0.97 (Appendix B). This leads to the assumption that parotid fluid can be utilized to reflect metabolic activity of this component.

5.6 Cholinesterase (Pseudocholinesterase)

No significant amount of pseudocholinesterase was found in over 60 parotid fluid samples analyzed. Several methods and modifications of existing techniques were utilized in an attempt to obtain values for parotid pseudocholinesterase. No suitable method was found. The effective level of pseudocholinesterase is below the sensitivity of the methods currently in use. It may be possible, however, to develop a technique to measure cholinesterase in parotid fluid. This would be a burdensome program and appears that pseudocholinesterase is not feasible for such measurement at the present time.

5.7 Vanillylmandelic Acid (VMA)

The levels of VMA in parotid fluid are below the levels of sensitivity of the current method utilized for the measurement of VMA. No VMA was found in any of the 15 samples tested. It would appear that VMA is not a feasible material to monitor in parotid fluid at this time.

5.8 Urea Nitrogen Levels in Parotid Fluid

Parotid urea-nitrogen concentrations reflect the blood urea-nitrogen values. The linear equation $Y = 14.7 + 0.8\ (X-9.87)$

where X = concentration of urea nitrogen in parotid fluid (mg%)

Y = concentration of urea nitrogen in blood (mg%)

expresses the relationship.

Linear regression analysis of the component reveals a correlation coefficient of 0.8 for the values of the various groups tested. The range of urea nitrogen in parotid fluid varies from a low of 3.0 mg% (normal) to a high of 53 mg% (hospital). See Appendix A. In evaluating the various groups for differentiation between groups the following populations can be distinguished:

1) Normal and post-exercise groups
2) Normal and hospitalized groups
3) Control and exercised groups
4) Control and post-exercise groups

Based on the fact that parotid values for urea nitrogen can predict blood values it should be possible to use parotid fluid to evaluate those metabolic conditions which are indicated by an elevated or decreased level of blood urea-nitrogen.

5.9 Protein Concentration in Parotid Fluid

Protein concentration in parotid fluid varies widely among individuals. A range from 35 mg% (marine group) to a high of 999 mg% (an alcoholic from the hospitalized group) was found. The range for mean values for the five groups studied is from a low of 131 mg% for the control group to a high of 234 mg% for the hospitalized group (Appendix A).

By applying the t-test, an estimation of the probability that the means of the various samples are from different populations was tabulated (Appendix B).

The populations that can be distinguished are:

1) Normal and control groups
2) Hospitalized and control groups
3) Hospitalized and exercise groups

The marine groups show an increase of 43% in the mean value post-exercise. However, when subjecting these two groups (control and post-exercise) to the t-test, there is only a probability of 0.9 that they are from different populations (Appendix B). A 0.95 or 0.99 probability would be more desirable in order to draw conclusions as to the feasibility of parotid fluid for biochemical monitoring. However, a probability of 0.9 at least may be interpreted as showing a tendency. Further studies on protein concentrations in parotid fluid would probably reveal whether or not exercise causes this rise.

5.10 Glucose Concentration in Parotid Fluid

Glucose concentration in parotid fluid is two orders of magnitude lower than that found in blood. The mean value of glucose in parotid fluid is approximately 1 mg%; however, values as high as 5.60 mg% and as low as 0.1 mg% were noted.

No distinction between the normal, hospitalized and control populations could be made statistically (Appendix B). A study of the marine population without stimulation (Appendix A) reveals an increase in glucose concentration

post-exercise of 39%. In the post exercise group using the lemon stimulant (an average of 7 stimulations over the test period) a highly significant increase of 177% was found.

Since the method of stimulation (Appendix F) was normally of this type it was necessary to establish that there was no leakage into the collection cup. Since the system requires a positive head pressure to move the fluid down the capillary tubing, if any leakage occurred it would be in an outward direction rather than inward. However, in order to substantiate this fact, citric acid was used as a stimulant. If any leakage inward was occurring, a change in pH of the parotid fluid should be noted. No change could be detected. A glucose tolerance test was performed. With the collector in place the subject was given a concentrated solution of glucose to injest (100 g diluted with 400 ml of H_2O), a concentration far above that normally used. No elevation in glucose was noted in the parotid fluid during or immediately after the ingestion period. One-half hour after the ingestion, the glucose level increased approximately 70% and continued to rise to a value of 8.2 mg%, an increase in level of 120% over the base line value of 3.4 mg%. The level of glucose returned to the baseline value by the second hour and dropped below baseline by three hours. These observations lead to the conclusion that values reported for glucose are due to metabolic activity rather than from external contamination.

An average of 1 to 2 lemon stimulants were used for stimulation purposes for the normal, hospitalized and control data. The sampling time was usually less than 20 minutes. By rigid control of the collection technique, a comparison of the various populations can be made (Appendix B).

It would appear that glucose values in parotid fluid can be utilized for studying the relationships of glucose metabolism under varying conditions. Sensitivity of the methodology for the measurement of glucose in parotid fluid has been developed to 1 part per million, although the method does require careful technique.

6.0 RECOMMENDATIONS:

In order to maximize the utility of parotid secretion and to predict the continuing capability of personnel operating space craft or high performance vehicles, additional information should be accumulated.

The following list of recommendations delineates the areas which at this time appear to be most essential to such an investigation.

1) Deviations of individual values from group means (Appendices A, D and E) are such that biological and physiological characterization can only be achieved by monitoring individual subjects for extended periods. Diurnal variations must be elucidated. A specific program calling for multi-samplings daily over a period of several months appears necessary to interpret parotid secretion in terms of physiological change on a select subject.

2) Variations in physical characteristics of parotid fluid such as conductivity and density might profitably be explored for biochemical relationships.

3) Examination of biochemical changes in other parotid components than those currently considered may be helpful in predicting physiological changes. Materials suggested for study include amylase, ketosteroids, selected protein fractions, and total carbohydrates.

4) Specific metabolic processes should be studied to correlate pathological and physiological changes with changing parotic fluid values, e.g. a study of elevated ammonia in hepatic insufficiency might be correlated with parotid ammonia levels.

APPENDIX A

DATA TABLES

COMPONENT CONCENTRATIONS FOR TEST POPULATIONS MEASURED UNDER QUIESCENT CONDITIONS

TEST	NORMAL					HOSPITALIZED					CONTROL (MARINES)				
	# Cases	Mean	S.D.	High	Low	No. Cases	Mean	S.D.	High	Low	No. Cases	Mean	S.D.	High	Low
PAROTID															
Flow ml/min	106	1.20	0.70	3.41	0.02	151	1.15	0.72	3.64	0.03	63	1.14	0.53	2.96	0.30
PO_2 mm Hg	10	95.0	33.1	142	46.0	10	87.2	16.2	110	60.0	19	81.2	17.8	113	52.0
PCO_2 mm Hg	10	31.8	7.4	44.0	21.0	10	33.6	4.9	40.0	25.0	22	37.8	6.8	51.0	24.0
HCO_3 mmoles/l	10	14.4	6.5	25.1	5.7	10	13.8	6.1	25.9	8.5	21	21.6	4.8	33.2	14.1
Urea-N mg%	31	9.2	2.3	13.3	3.0	32	12.1	10.5	53.6	4.3	16	8.8	2.2	12.0	4.6
NH_3-N mg%	8	127	41.3	185	65.0	7	142	68.2	267	67.0	20	114	47.4	203	45.0
Ca meq/l	67	1.7	0.3	2.4	1.0	51	1.73	0.50	3.6	1.00	16	1.65	0.23	2.00	1.30
Prot. mg%	49	201	144	665	66.0	42	234	186	999	51.0	13	131	79.8	294	35.0
Glucose mg%	28	0.95	0.73	3.60	0.30	41	0.98	1.05	5.60	0.10	15	1.37	1.05	4.20	0.10
BLOOD															
PO_2 mm Hg	10	36.7	17.3	78.0	18.0	10	32.5	7.7	44.0	20.0	20	25.2	6.2	42.0	17.0
PCO_2 mm Hg	10	43.4	5.4	54.0	36.0	10	46.2	5.5	55.0	35.0	20	47.6	8.3	58.0	22.0
HCO_3 mmoles/l	10	24.9	5.1	36.3	18.9	10	21.2	2.7	25.0	16.8	20	19	25.5	46.8	19.6
Urea-N mg%	18	13.8	2.8	18.5	8.6	17	14.4	4.5	23.7	7.2	14	14.0	2.7	19.0	9.7
NH_3-N mg%	7	145	26.7	196	118	0	0	0	0	0	18	127	24.4	192	94.0
Ca. meq/l	17	4.6	0.18	4.9	4.0	24	4.7	0.48	5.70	3.10	14	4.8	0.17	5.1	4.3
Prot. g%	17	7.0	0.35	7.5	6.4	19	7.1	0.60	8.40	6.0	11	7.0	0.25	7.5	6.7
Glucose mg%	14	83.4	14.9	105	3.0	20	95.1	19.7	147	1.00	11	97.3	13.9	123	77.0
URINE															
Ca. meq/l	19	10.5	5.8	21.0	2.3	38	5.71	4.74	19.0	0.20	0	0	0	0	0
Glucose mg%	6	14.0	28.0	74.0	0.8	20	12.8	30.0	99.0	0.50	7	2.0	0.55	2.8	1.4
Protein mg%	6	2.7	1.9	5.0	1.0	8	59.7	141	415	1.00	16	0.3	0.5	2.0	1.0

CONTROL GROUP COMPONENT CONCENTRATIONS PERTAINING TO PHYSICAL EXERTION

Test	Pre-Exercise					During Exercise						Post-Exercise					
PAROTID	# Cases	Mean	S.D.	High	Low	# Cases	Mean	S.D.	High	Low	% Change	# Cases	Mean	S.D.	High	Low	% Change
Flow ml/min	63	1.14	0.53	2.96	0.30	64	0.71	0.43	2.20	0.02	-38%	64	0.84	0.46	2.12	0.09	-26%
PO_2 mm Hg	19	81.2	17.8	113	52.0	18	66.6	20.3	108	34.0	-18%	19	55.6	20.8	108	30.0	-32%
PCO_2 mm Hg	22	37.8	6.8	51.0	24.0	21	36.5	8.09	47.0	17.0	-3.5%	22	35.3	6.85	47.0	22.0	-6.6%
HCO_3 m moles/l	21	21.6	4.8	33.2	14.1	20	19.1	5.8	34.	8.9	-12%	20	19.5	4.9	29.8	12.6	-10.%
Urea N mg%	16	8.8	2.2	12.0	4.6	16	10.1	2.29	13.3	5.10	+15%	16	10.3	2.43	15.0	5.80	+18%
NH_3 N ug%	20	114	47.4	203	45.0	15	179	91.7	354.0	64.0	+4.8%	11	119.	36.4	172	74.0	+5.1%
Ca Meq/l	16	1.65	0.23	2.00	1.30	14	1.64	0.25	2.10	1.20	-.61%	14	1.84	0.24	2.20	1.40	+12%
Prot. mg%	13	131	79.8	294	35.0	13	139	66.5	272	40.0	+6.3%	15	188	106	421	66.0	+44%
Glucose mg%	15	1.37	1.05	4.20	0.10	15	1.86	1.50	5.40	0.50	+36%	16	3.79	2.34	8.40	0.90	+177%
BLOOD																	
PO_2 mm Hg	20	25.2	6.2	42.0	17.0							20	27.5	7.83	43.0	18.0	+9.1%
PCO_2 mm Hg	20	47.6	8.3	58.0	22.0							20	42.9	7.33	52.0	17.0	-9.8%
HCO_3 m moles/l	20	19	25.5	46.8	19.6							19	22.1	6.49	40.8	1.90	-16%
Urea N mg%	14	14.0	2.7	19.0	9.7							14	13.5	2.98	19.3	8.90	-3.6%
NH_3 N ug%	18	127	24.4	192	94.0							17	125	32.2	180	78.0	-1.1%
Ca Meq/l	14	4.8	0.17	5.1	4.3							14	4.86	0.46	5.30	3.10	+1.7%
Prot g%	11	7.1	0.25	7.5	6.7							10	7.58	0.36	8.20	7.10	+6.3%
Glucose mg%	11	97.3	13.9	123	77.0							14	90.6	12.2	116	73.0	-6.8%
Urine																	
Ca Meq/l	0	0.0	0.0	0.0	0.0							0	0.	0.	--	--	--
Glucose mg%	7	2.0	0.55	2.8	1.4							8	1.72	0.59	2.50	0.90	-12%
Prot mg%	16	0.3	0.5	2.0	1.0							12	3.13	5.28	22.0	1.00	+94%

MARINES WITHOUT STIMULATION

Test	No. Cases	Pre-exercise			Exercise				Post-exercise			
		High	Low	Mean	High	Low	Mean	% Mean Change	High	Low	Mean	% Mean Change
Parotid												
Urea mg %	7	21.2	8.8	15.0	27.1	16.1	19.7	31.3	19.3	8.9	16.0	6.7
Calcium meq/l	3	2.4	1.9	2.2	3.2	2.0	2.6	18.2	3.5	2.1	2.7	22.7
Protein mg %	3	267	125	189	286	169	217	14.8	562	166	326	72.5
Glucose mg%	3	2.5	0.6	1.4	3.6	0.7	2.1	50	2.4	1.2	1.9	35.7
Blood												
Urea mg%	7	18.8	11.5	14.1					17.9	11.2	13.8	2.1
NH_3 ug%	4	126	102	114					137	88	115	.9
Protein g%	3	7.2	6.8	7.0					7.7	7.2	7.4	5.7
Glucose mg%	3	146	94	122					131	94	112	8.2
Urine												
Calcium meq/l	3	6.6	5.4	6.0					10.9	7.3	9.1	51.6
Glucose mg%	3	3.3	2.0	2.7					9.0	1.1	4.1	51.8

COMPARISON OF MEAN VALUES WITH/WITHOUT
STIMULATION - MARINES ONLY

	Pre-Exercise		Exercise		Post-Exercise	
	With	Without	With	Without	With	Without
Parotid						
Urea mg%	8.8	15	10.1	19.7	10.3	16.0
Ca meq/l	1.6	2.2	1.6	2.6	1.8	2.7
Prot. mg%	131	189	139	217	188	326
Glu. mg%	1.4	1.4	1.9	2.1	3.8	1.9
Blood						
Urea mg%	14.0	14.1			13.5	13.8
NH_3 ug%	127	114			125	115
Prot. g%	7.1	7.0			7.6	7.4
Glu. mg%	97	122			91	112
Urine						
Ca meq/l	—	6.0			—	9.1
Glu. mg%	2.0	2.7			1.7	4.1

APPENDIX B

STATISTICAL TABLES

PARTIAL PRESSURE OF CARBON DIOXIDE IN PAROTID FLUID

POPULATIONS STUDIED

	(1) NORMAL	(2) HOSPITAL	(3) CONTROL	(4) EXERCISE	(5) POST-EXERCISE
NORMAL		0.7	0.99	0.975	0.8
HOSPITAL	0.7		0.95	0.8	0.7
CONTROL	0.99	0.95		0.7	0.8
EXERCISE	0.975	0.8	0.7		0.7
POST-EXERCISE	0.8	0.7	0.8	0.7	

The probability is presented that the mean values for the parotid parameter are from different populations. A probability of 0.99 or greater is considered highly significant; 0.95 significant; and 0.90 showing only a trend. Any probability below 0.90 is inconclusive.

PARTIAL PRESSURE OF OXYGEN IN PAROTID FLUID

POPULATIONS STUDIED

	(1) NORMAL	(2) HOSPITAL	(3) CONTROL	(4) EXERCISE	(5) POST-EXERCISE
NORMAL		0.8	0.95	0.995	0.999
HOSPITAL	0.8		0.8	0.995	0.999
CONTROL	0.95	0.8		0.975	0.999
EXERCISE	0.995	0.995	0.975		0.95
POST-EXERCISE	0.999	0.999	0.999	0.95	

The probability is presented that the mean values for the parotid parameter are from different populations. A probability of 0.99 or greater is considered highly significant; 0.95 significant; and 0.90 showing only a trend. Any probability below 0.90 is inconclusive.

BICARBONATE IN PAROTID FLUID

POPULATIONS STUDIED

	(1) NORMAL	(2) HOSPITAL	(3) CONTROL	(4) EXERCISE	(5) POST-EXERCISE
NORMAL		0.5	0.999	0.975	0.975
HOSPITAL	0.5		0.995	0.995	0.995
CONTROL	0.999	0.995		0.9	0.9
EXERCISE	0.975	0.995	0.9		0.6
POST-EXERCISE	0.975	0.995	0.9	0.6	

The probability is presented that the mean values for the parotid parameter are from different populations. A probability of 0.99 or greater is considered highly significant; 0.95 significant; and 0.90 showing only a trend. Any probability below 0.90 is inconclusive.

AMMONIA IN PAROTID FLUID

POPULATIONS STUDIED

	(1) NORMAL	(2) HOSPITAL	(3) CONTROL	(4) EXERCISE	(5) POST-EXERCISE
NORMAL		0.7	0.7	0.9	0.6
HOSP...	0.7		0.8	0.8	0.8
CONTROL	0.7	0.8		0.975	0.6
EXERCISE	0.9	0.8	0.975		0.95
POST-EXERCISE	0.6	0.8	0.6	0.95	

The probability is presented that the mean values for the parotid parameter are from different populations. A probability of 0.99 or greater is considered highly significant; 0.95 significant; and 0.90 showing only a trend. Any probability below 0.90 is inconclusive.

UREA-NITROGEN IN PAROTID FLUID

POPULATIONS STUDIED

	(1) NORMAL	(2) HOSPITAL	(3) CONTROL	(4) EXERCISE	(5) POST-EXERCISE
NORMAL		0.975	0.800	0.900	0.950
HOSPITAL	0.975		0.900	0.800	0.800
CONTROL	0.800	0.900		0.950	0.950
EXERCISE	0.900	0.800	0.950		0.600
POST-EXERCISE	0.950	0.800	0.950	0.600	

The probability is presented that the mean values for the parotid parameter are from different populations. A probability of 0.99 or greater is considered highly significant; 0.95 is significant; and 0.90 showing only a trend. Any probability below 0.90 is inconclusive.

PROTEIN IN PAROTID FLUID

POPULATIONS STUDIED

	(1) NORMAL	(2) HOSPITAL	(3) CONTROL	(4) EXERCISE	(5) POST-EXERCISE
NORMAL		0.8	0.95	0.9	0.6
HOSPITAL	0.8		0.95	0.95	0.8
CONTROL	0.95	0.95		0.6	0.9
EXERCISE	0.9	0.95	0.6		0.8
POST-EXERCISE	0.6	0.8	0.9	0.8	

The probability is presented that the mean values for the parotid parameter are from different populations. A probability of 0.99 or greater is considered highly significant; 0.95, significant; and 0.90 showing only a trend. Any probability below 0.90 is inconclusive.

CALCIUM IN PAROTID FLUID

POPULATIONS STUDIED

	(1) NORMAL	(2) HOSPITAL	(3) CONTROL	(4) EXERCISE	(5) POST-EXERCISE
NORMAL		0.7	0.7	0.7	0.9
HOSPITAL	0.7		0.8	0.8	0.9
CONTROL	0.7	0.8		0.5	0.975
EXERCISE	0.7	0.8	0.5		0.975
POST-EXERCISE	0.9	0.9	0.975	0.975	

The probability is presented that the mean values for the parotid parameter are from different populations. A probability of 0.99 or greater is considered highly significant; 0.95 is significant; and 0.90 showing only a trend. Any probability below 0.90 is inconclusive.

GLUCOSE IN PAROTID FLUID

POPULATIONS STUDIED

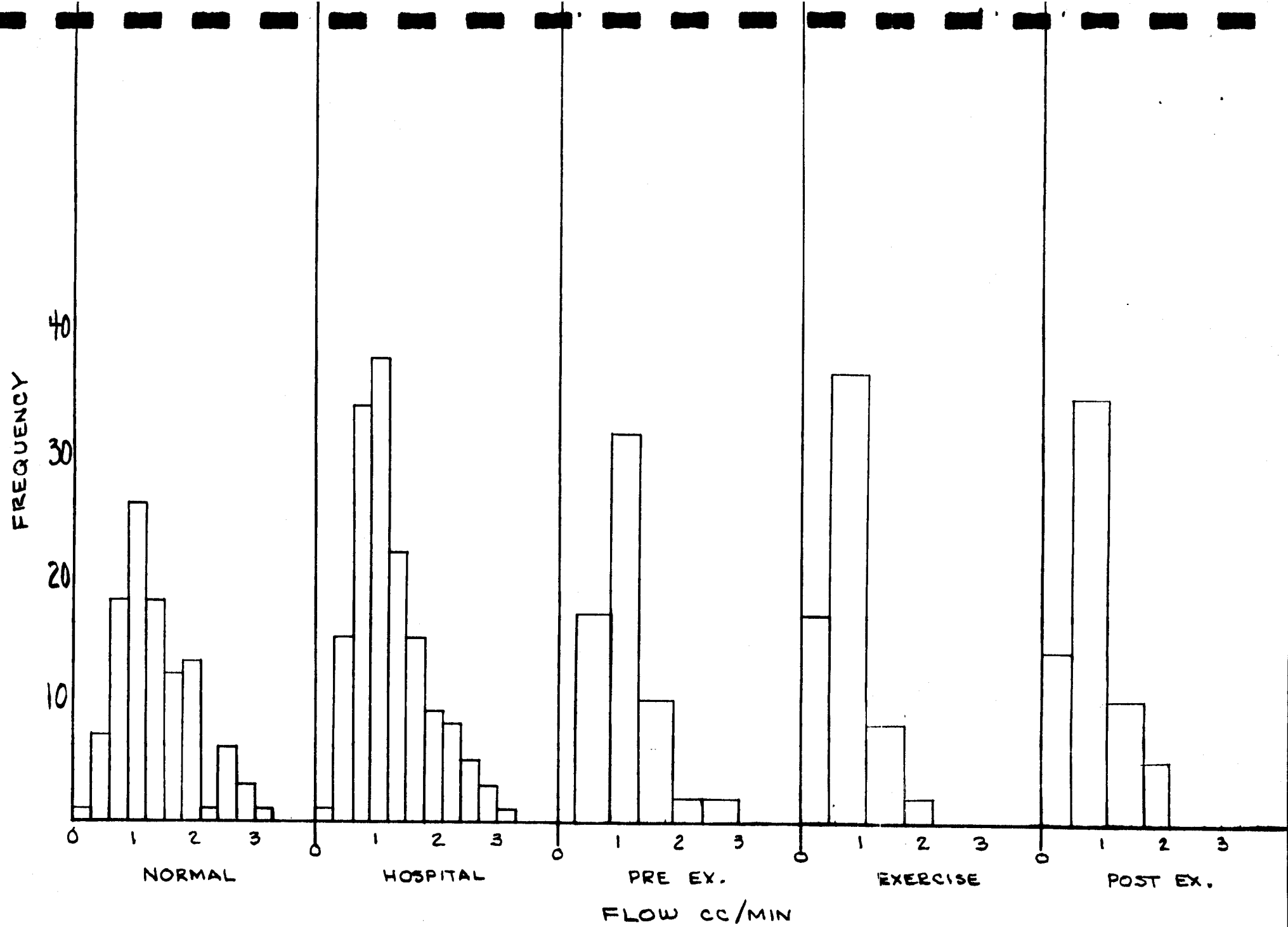
	(1) NORMAL	(2) HOSPITAL	(3) CONTROL
NORMAL		0.5	0.9
HOSPITAL	0.5		0.9
CONTROL (Marines)	0.9	0.9	

The probability is presented that the mean values for the parotid parameter are from different populations. A probability of 0.99 or greater is considered highly significant; 0.95, significant; and 0.90 showing only a trend. Any probability below 0.90 is inconclusive.

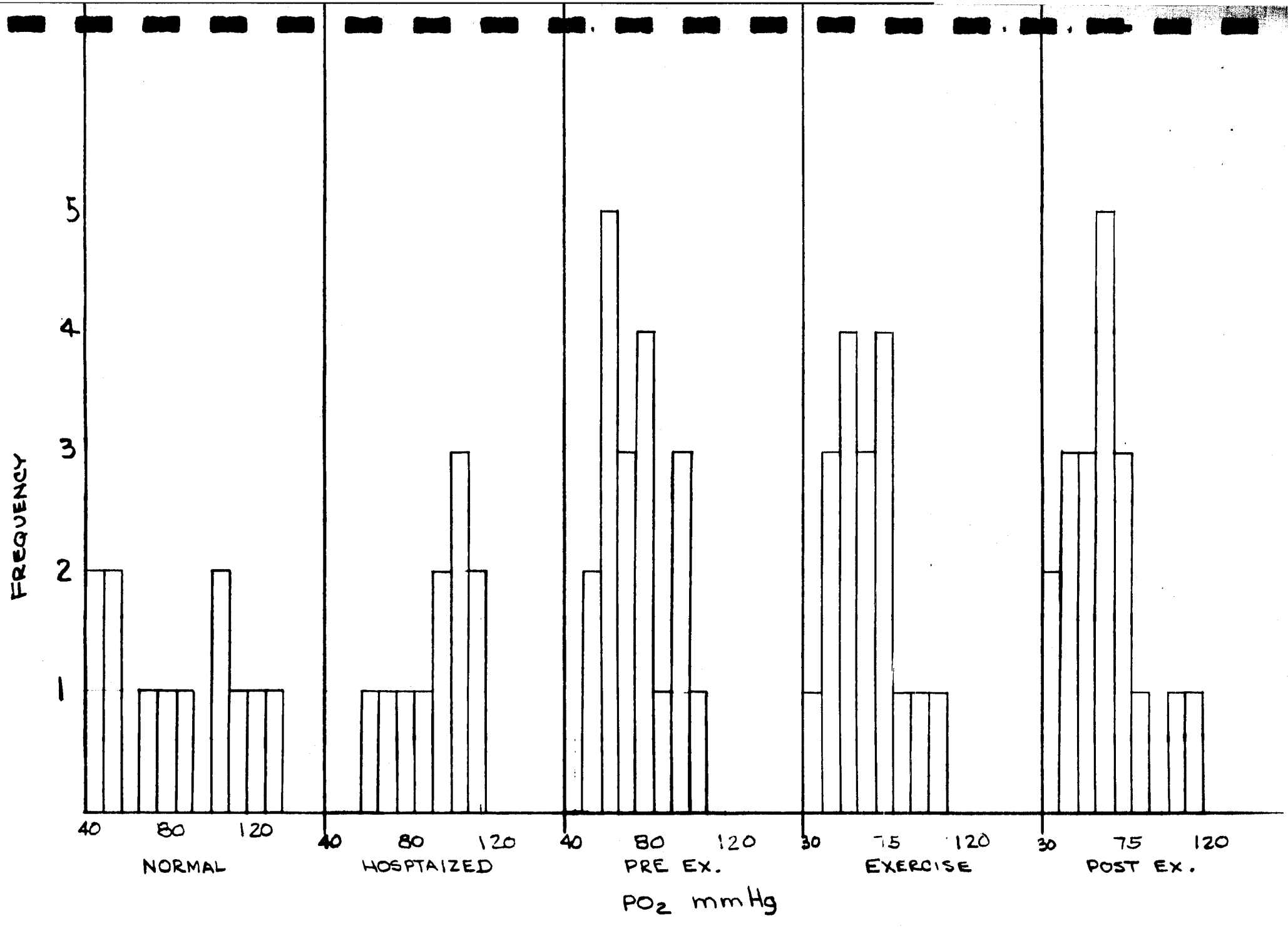
APPENDIX C

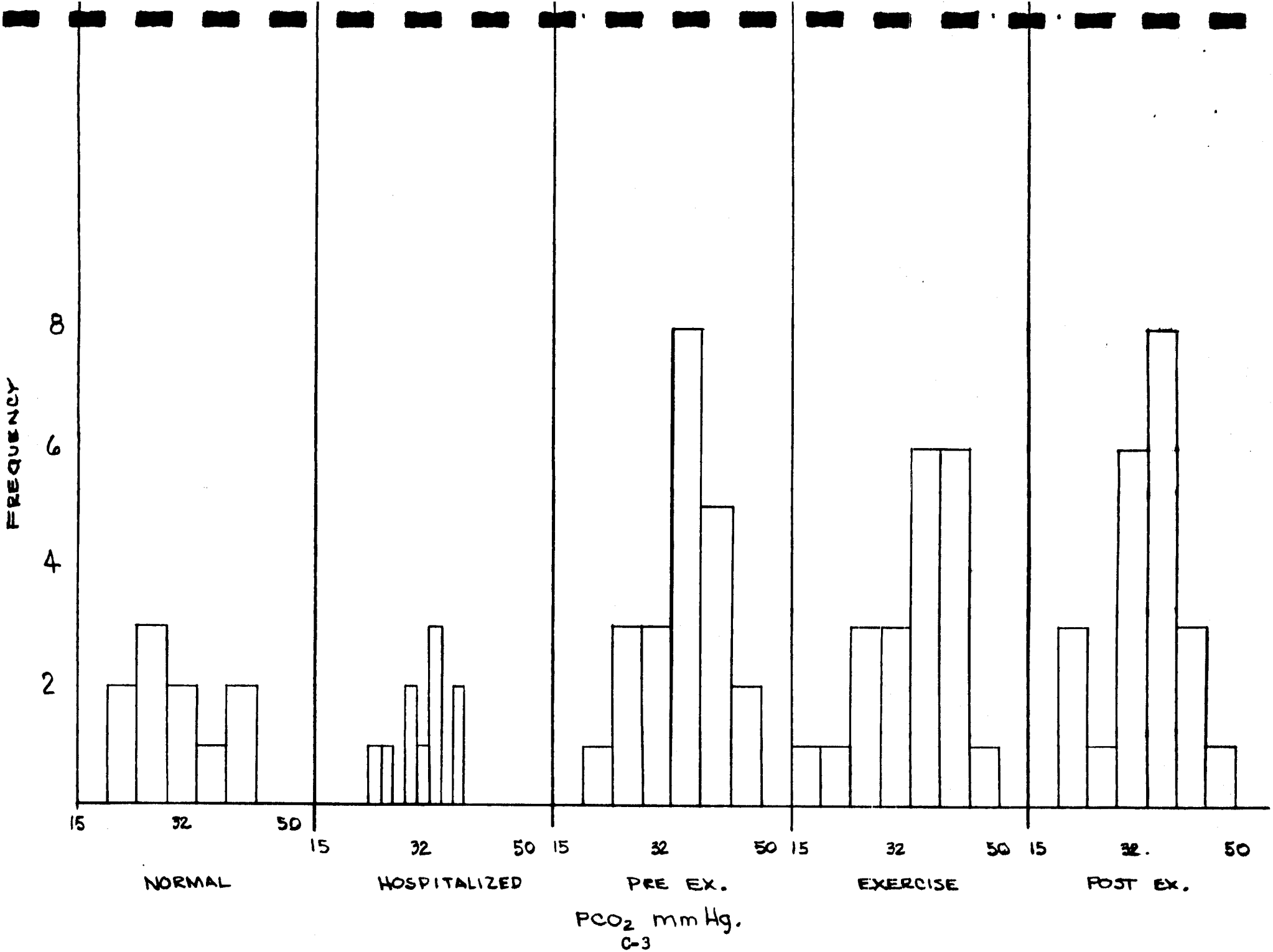
HISTOGRAMS OF PAROTID COMPONENTS

FOR ALL TEST POPULATIONS

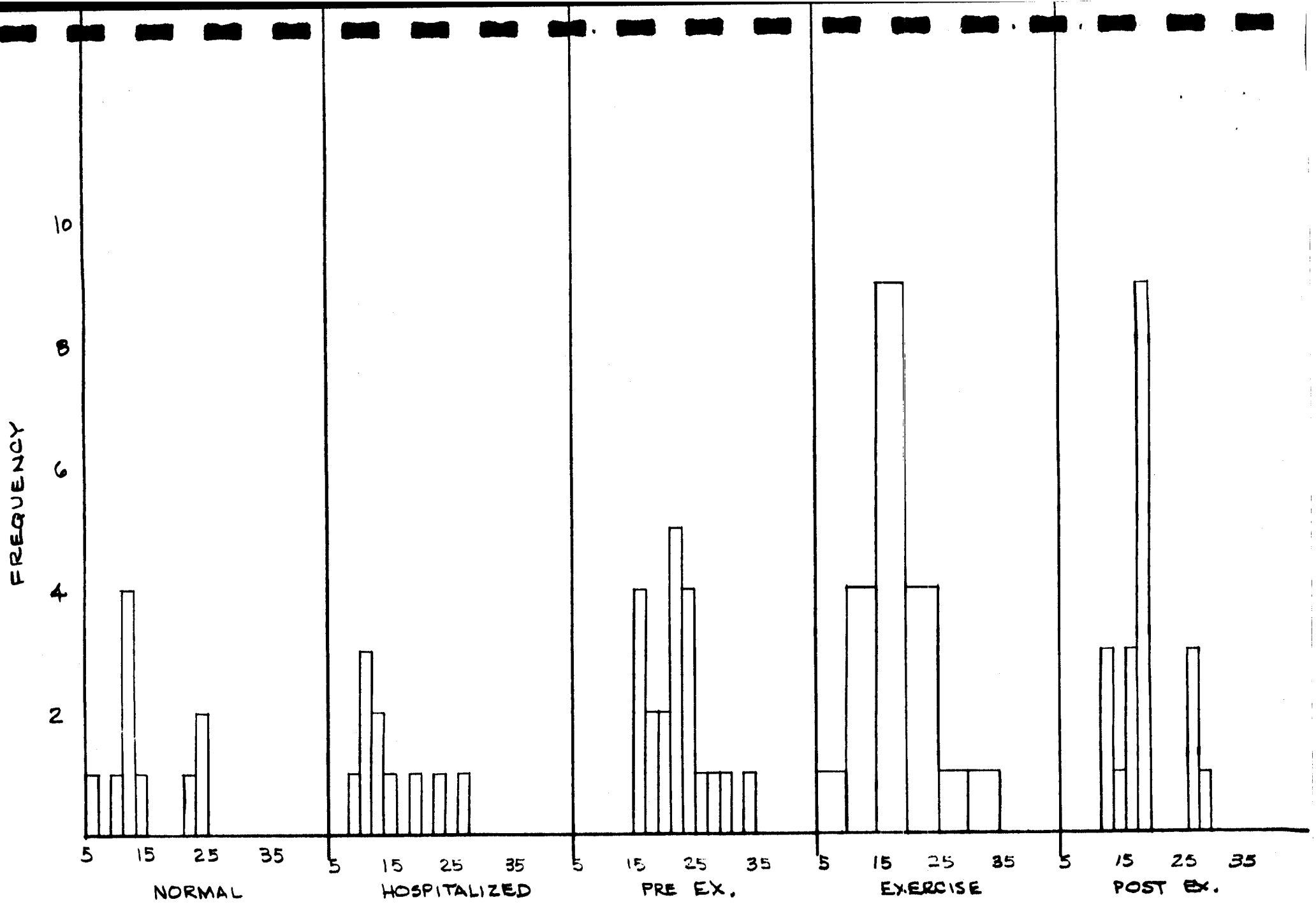

FREQUENCY
40
30
20
10
0 1 2 3
NORMAL
HOSPITAL
PRE EX.
EXERCISE
POST EX.
FLOW CC/MIN

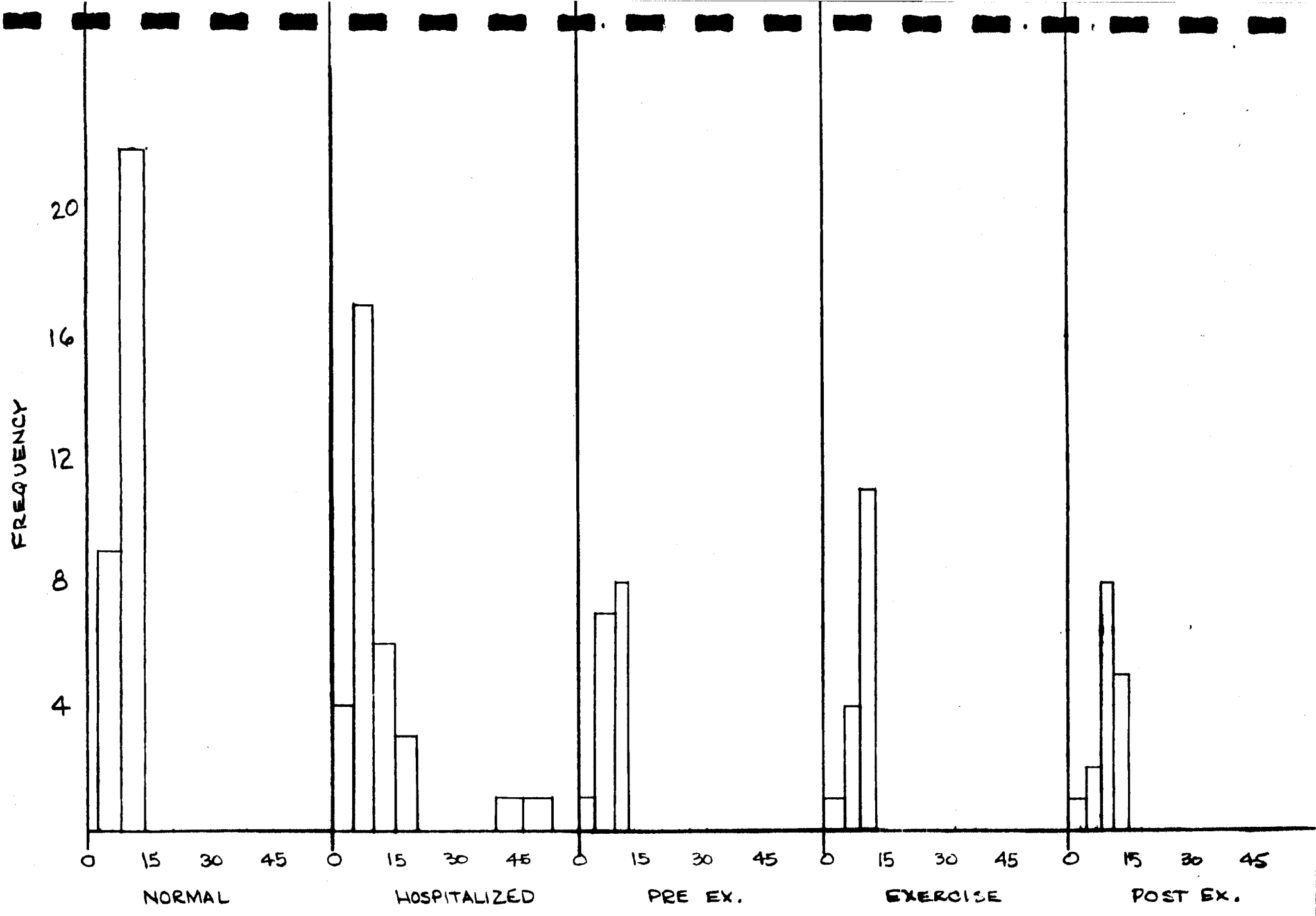
C-1


FREQUENCY
5
4
3
2
1
40
80
120
NORMAL
40
80
120
HOSPTAIZED
40
80
120
PRE EX.
30
75
120
EXERCISE
30
75
120
POST EX.
PO2 mmHg

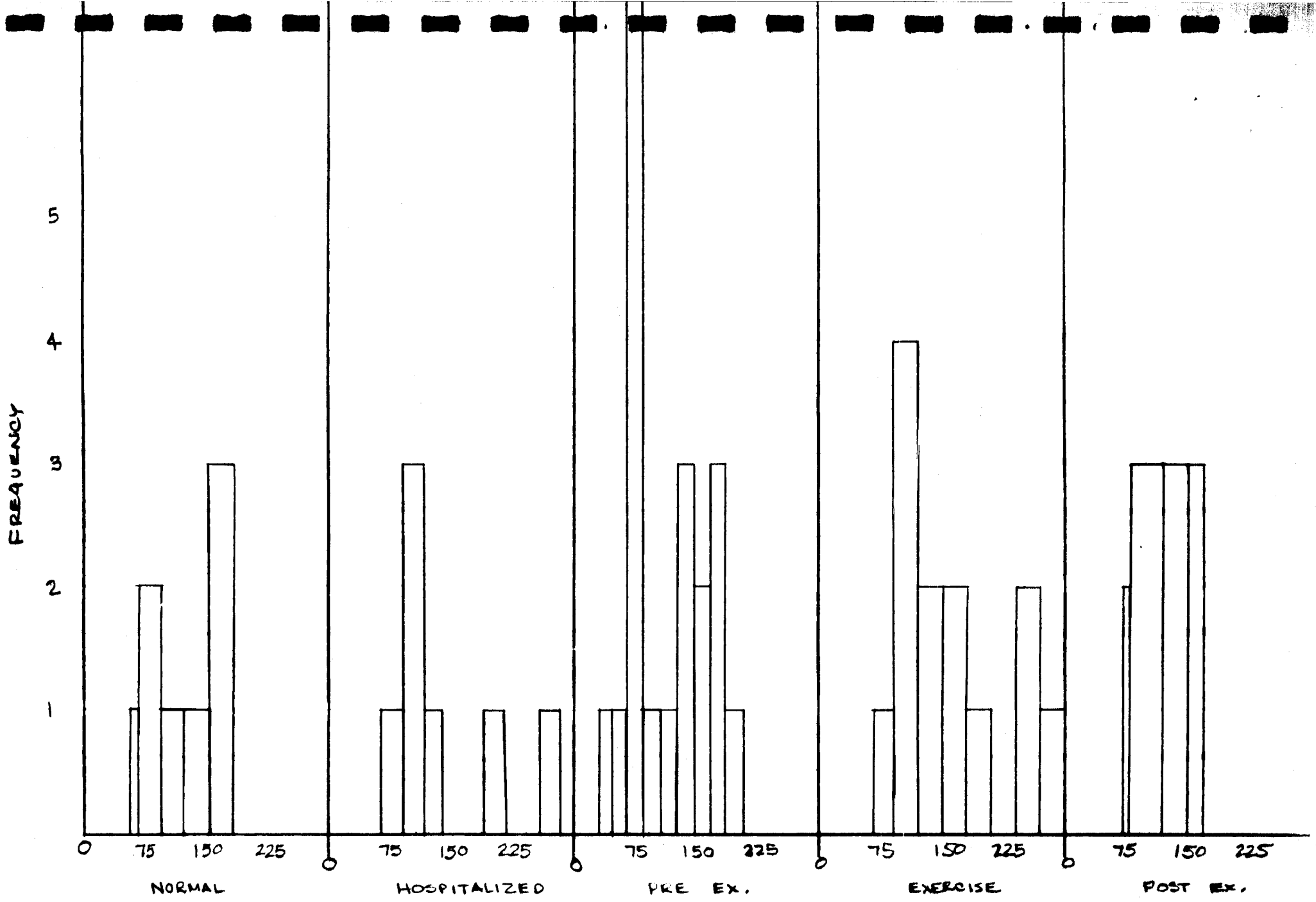

FREQUENCY
8
6
4
2
15
32
50
NORMAL
HOSPITALIZED
PRE EX.
EXERCISE
POST EX.
PCO2 mm Hg.

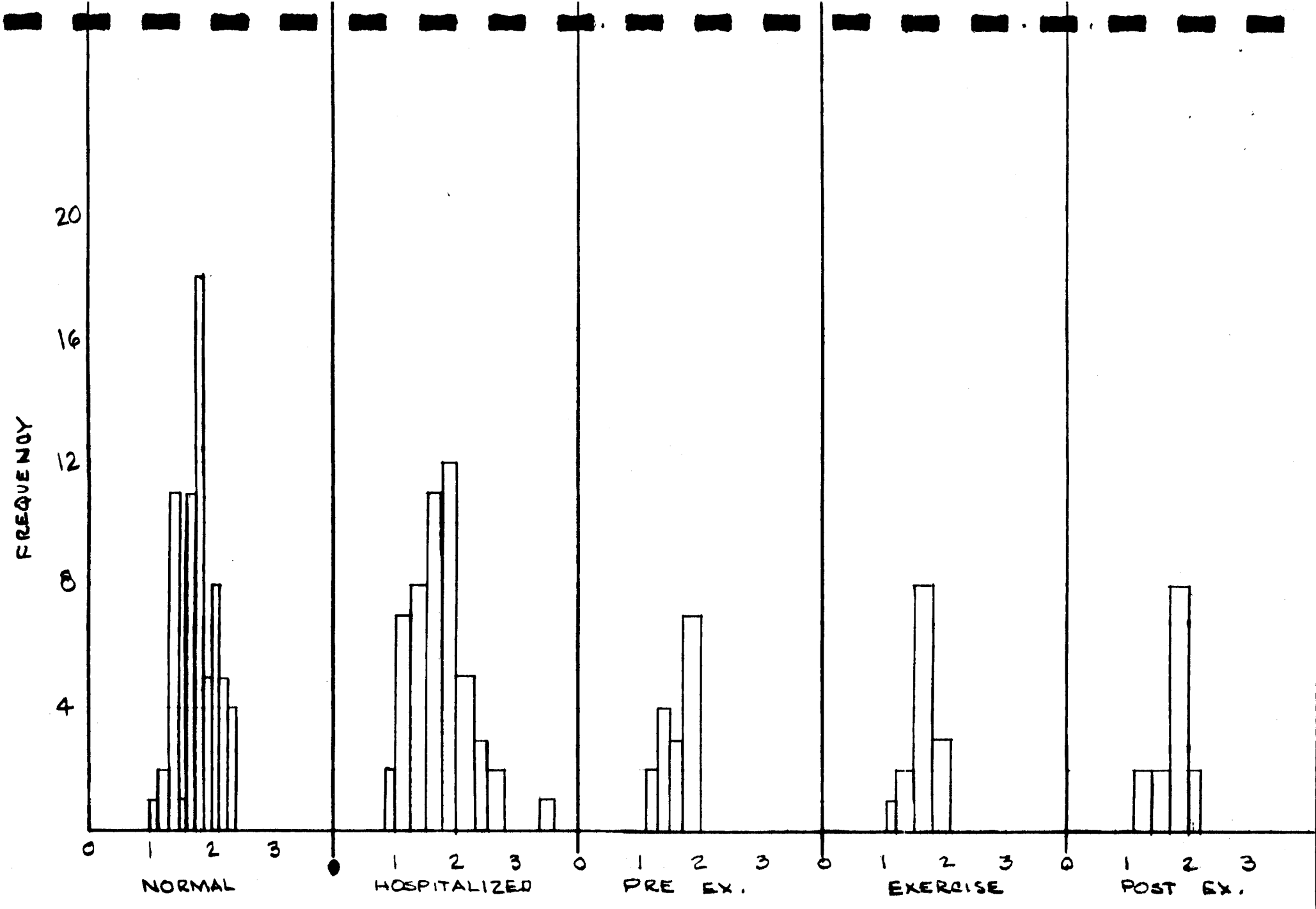
C-3

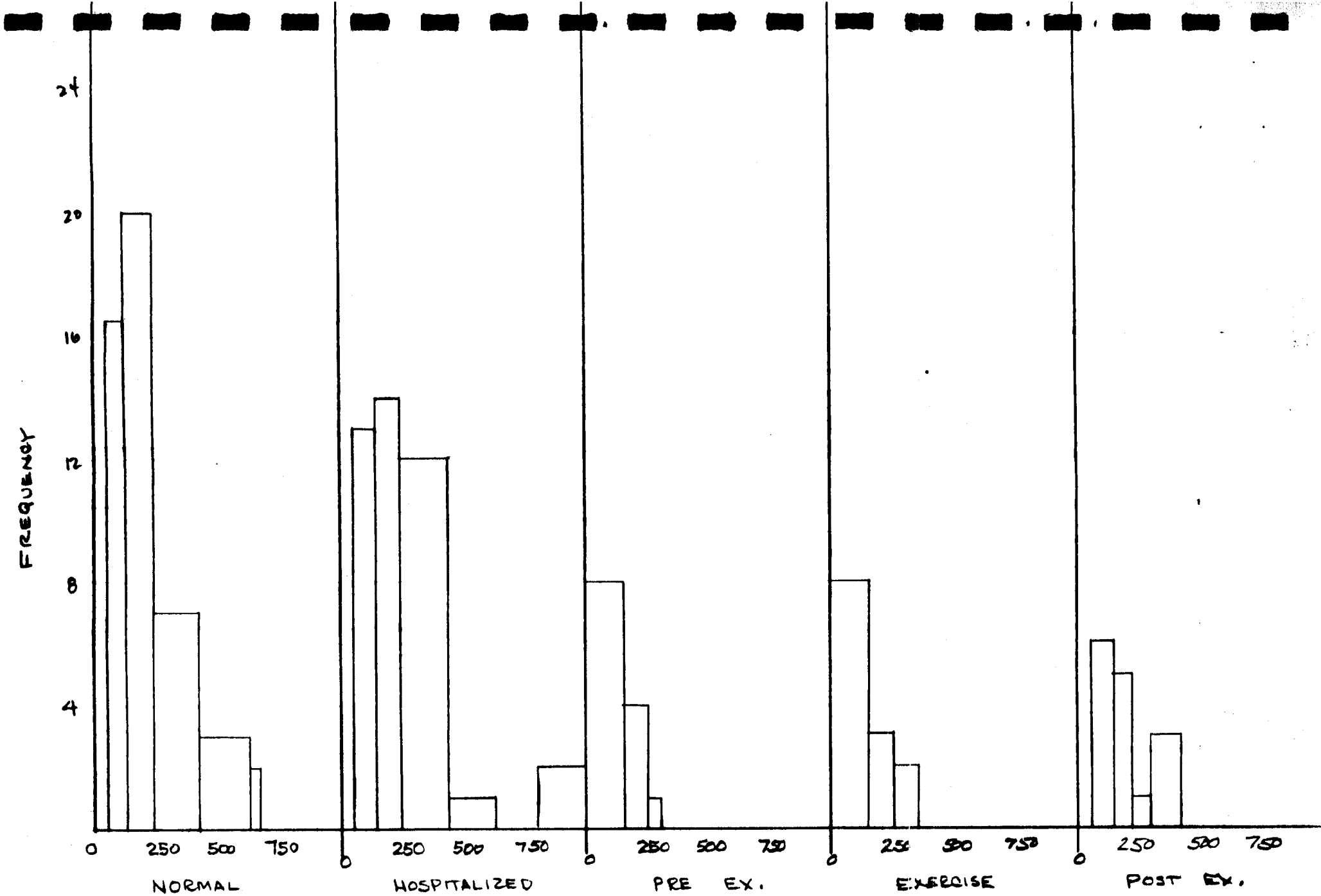

FREQUENCY
10
8
6
4
2
5 15 25 35
NORMAL
5 15 25 35
HOSPITALIZED
5 15 25 35
PRE EX.
5 15 25 35
EXERCISE
5 15 25 35
POST EX.
HCO3 mmoles/l


FREQUENCY
20
16
12
8
4
0
15
30
45
NORMAL
HOSPITALIZED
PRE EX.
EXERCISE
POST EX.
UREA - N mg %

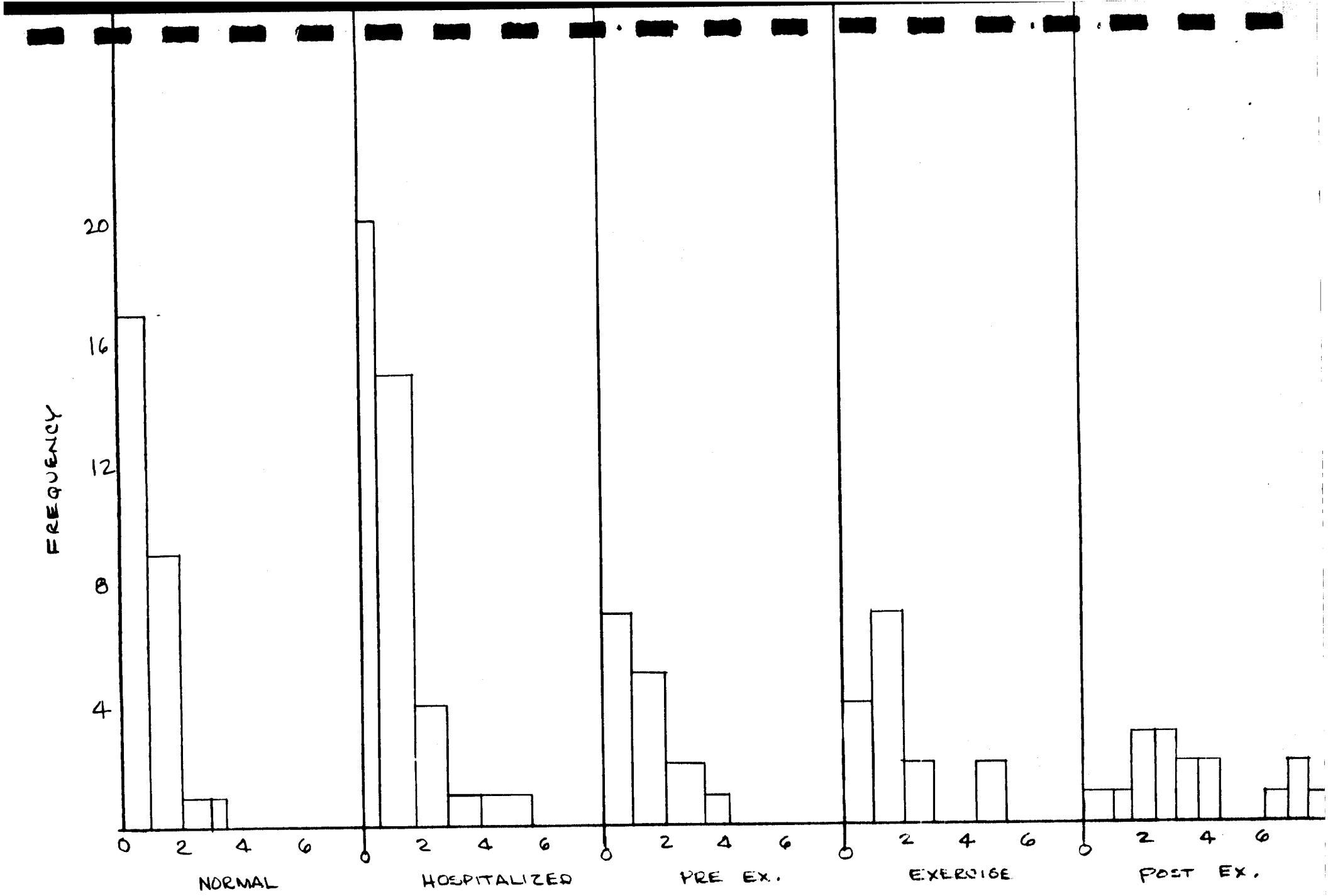
C-5


FREQUENCY
5
4
3
2
1
0
75
150
225
NORMAL
HOSPITALIZED
PRE EX.
EXERCISE
POST EX.
NH₃ - N mg%


FREQUENCY
20
16
12
8
4
0 1 2 3
NORMAL
1 2 3
HOSPITALIZED
0 1 2 3
PRE EX.
0 1 2 3
EXERCISE
0 1 2 3
POST EX.
CALCIUM MEQ/L

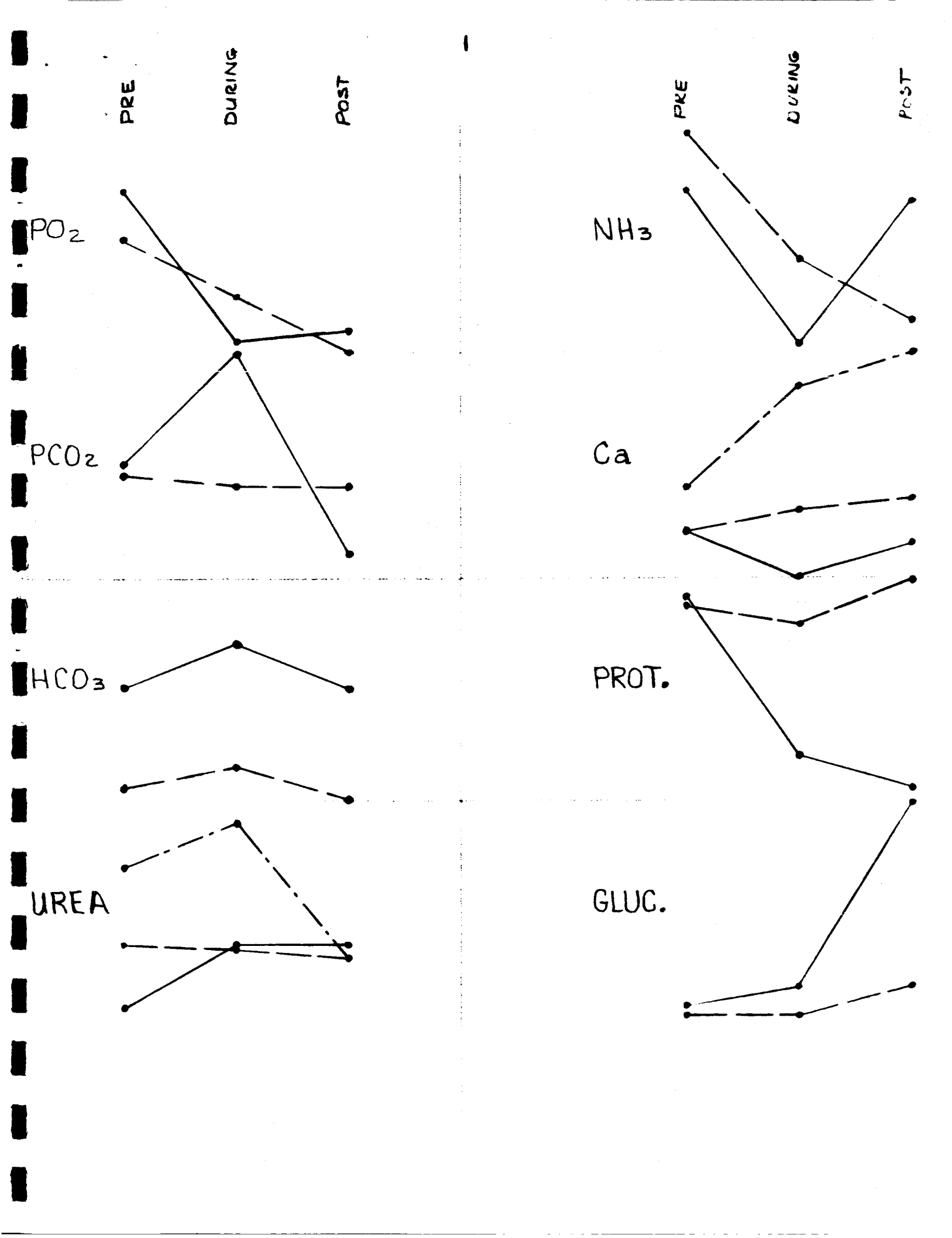

FREQUENCY
24
20
16
12
8
4
0
250
500
750
NORMAL
HOSPITALIZED
PRE EX.
EXERCISE
POST EX.

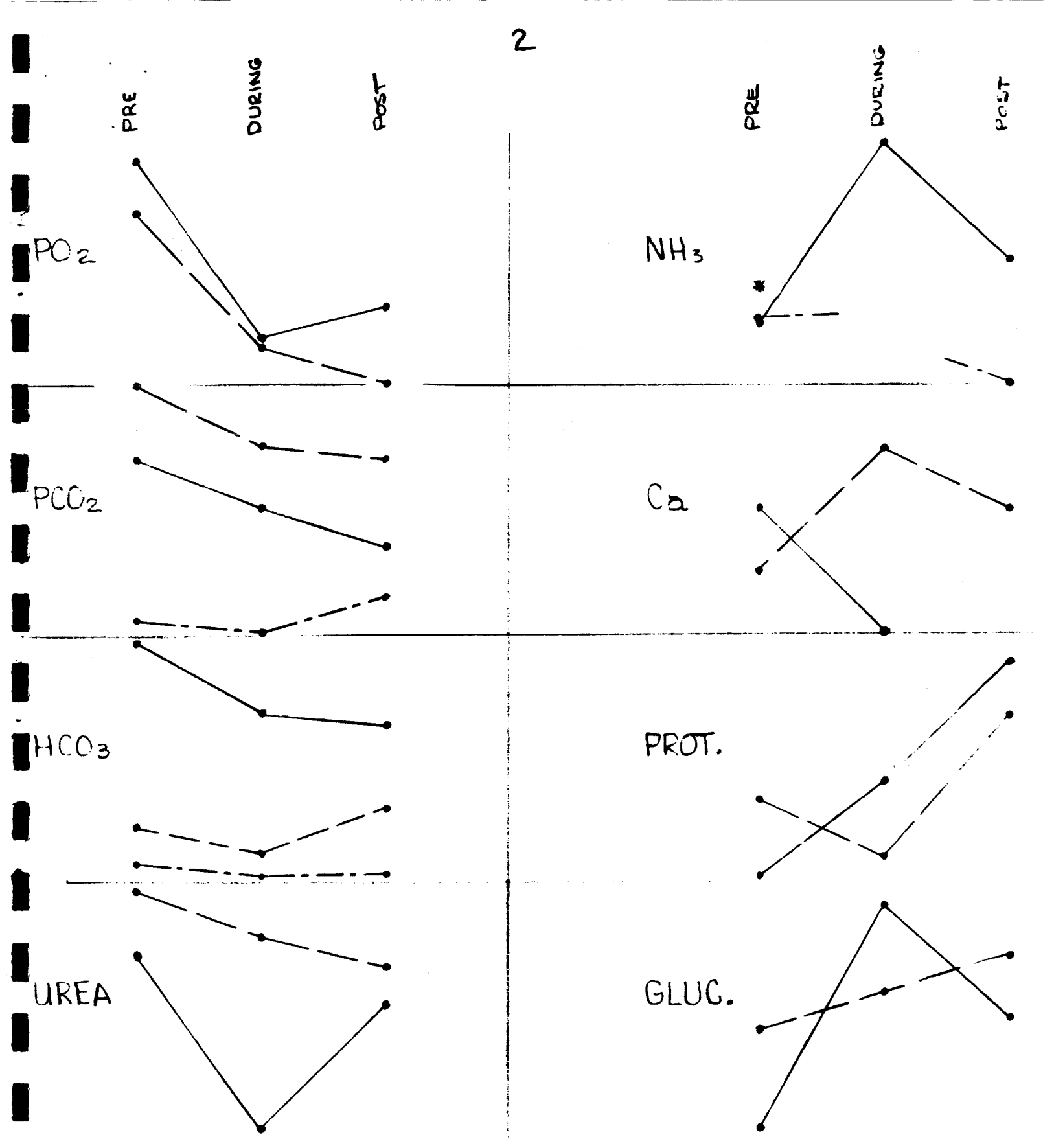
PROTEIN mg %

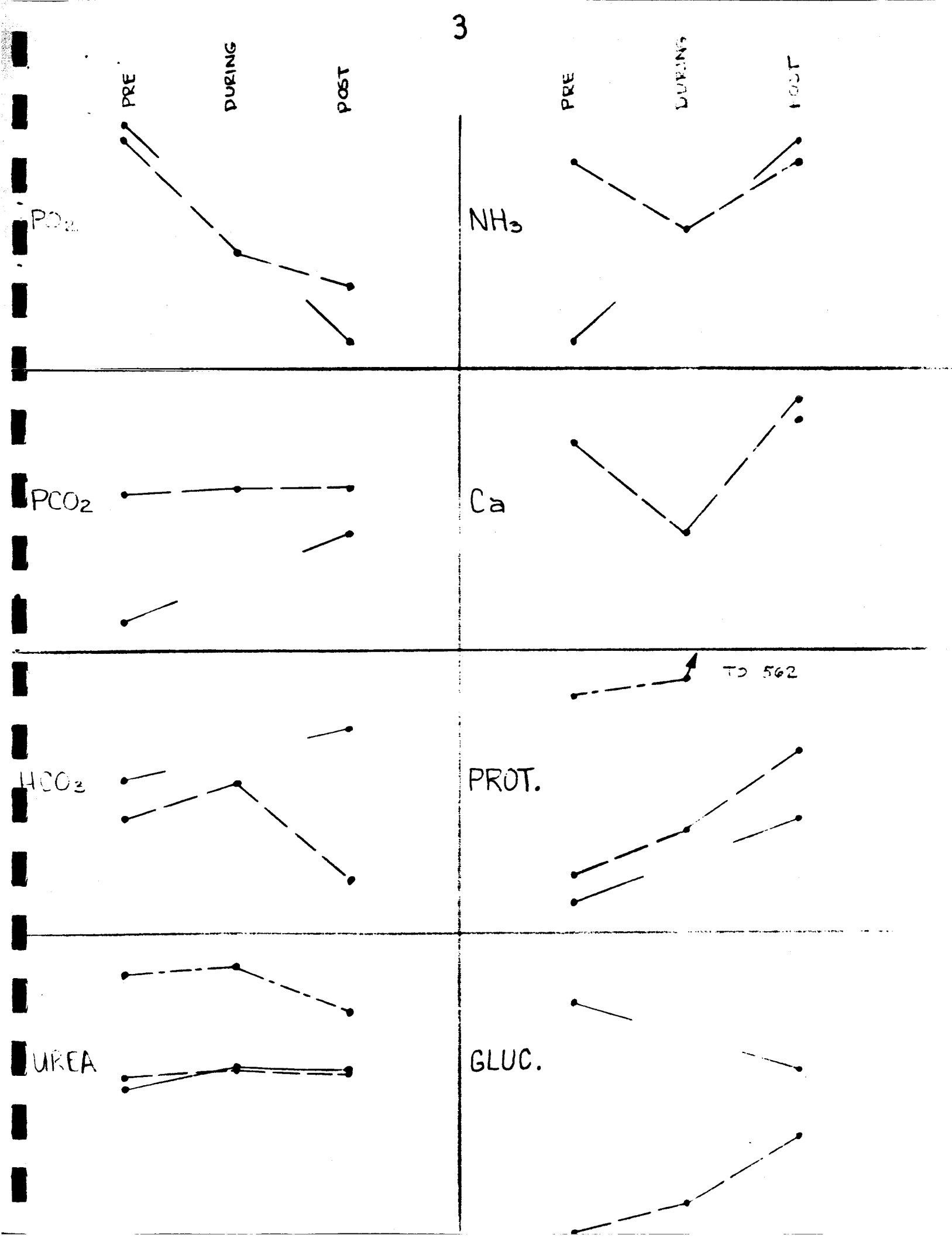

FREQUENCY
20
16
12
8
4
0 2 4 6
NORMAL
2 4 6
HOSPITALIZED
2 4 6
PRE EX.
2 4 6
EXERCISE
2 4 6
POST EX.
GLUCOSE mg %

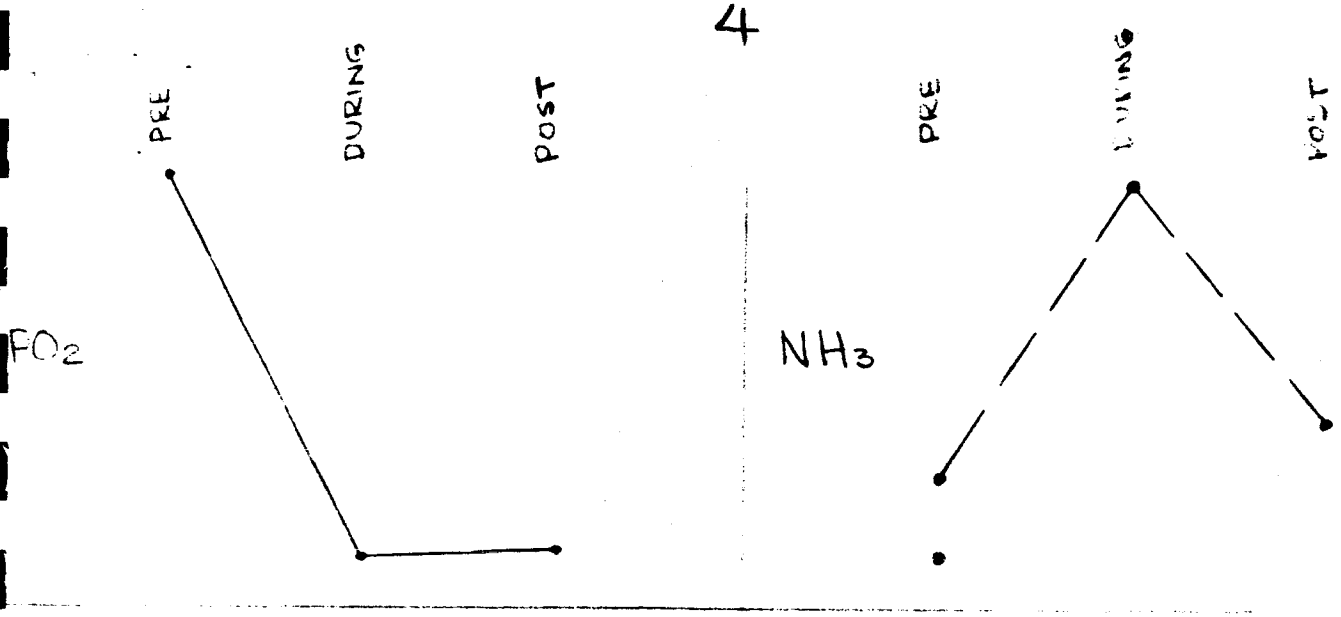
APPENDIX D

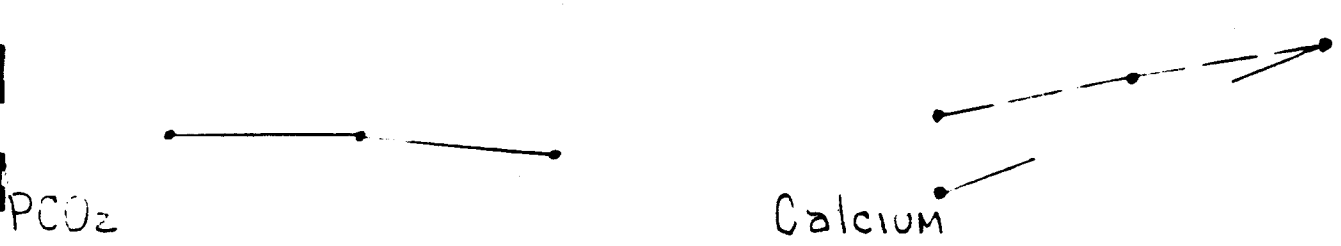
Graphs showing the variation of monitored parameters for each individual in the control group with repeated testing. Parotid values shown.

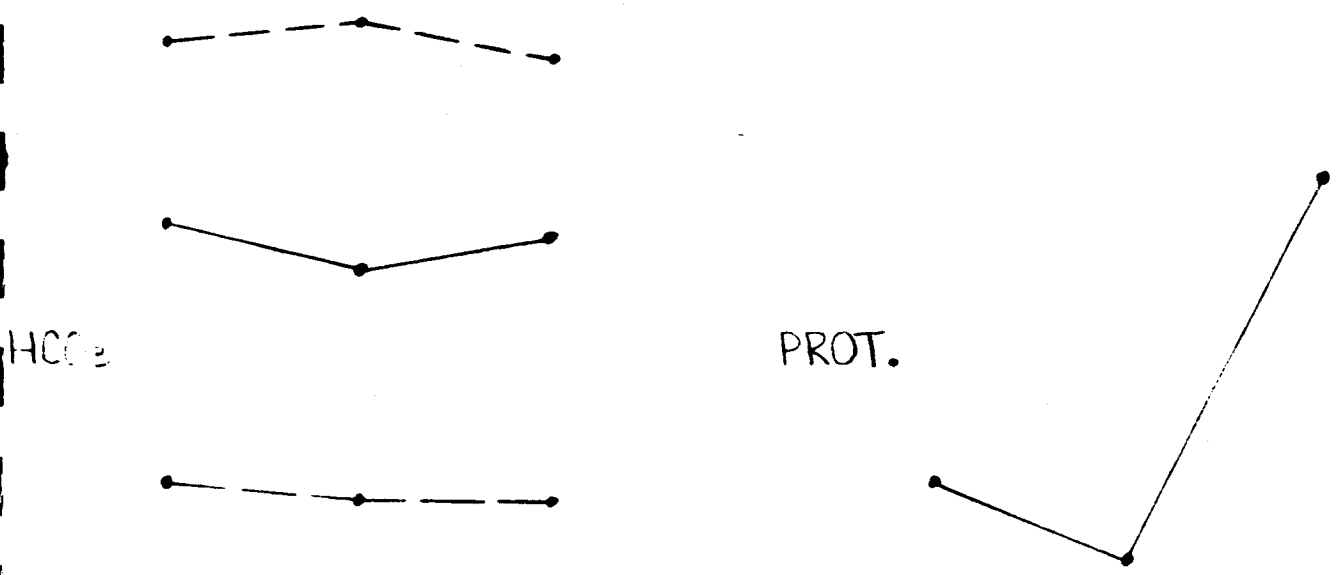

PRE
DURING
POST
PO_2
PCO_2
HCO_3
UREA
NH_3
Ca
PROT.
GLUC.

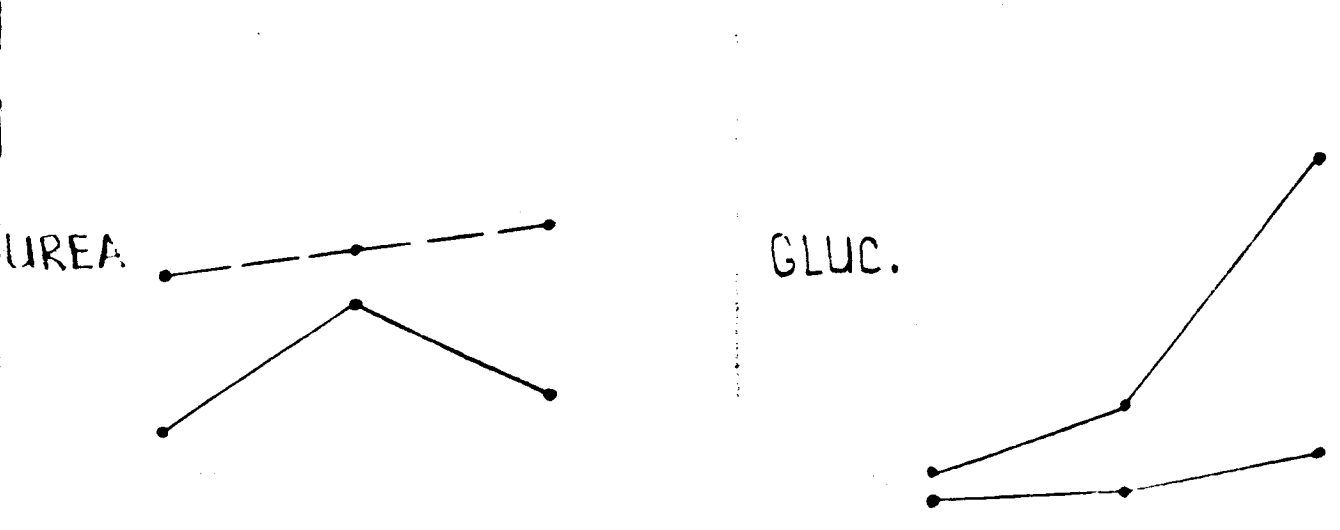

PRE
DURING
POST
PO_2
PCO_2
HCO_3
UREA
PRE
DURING
POST
NH_3
Ca
PROT.
GLUC.

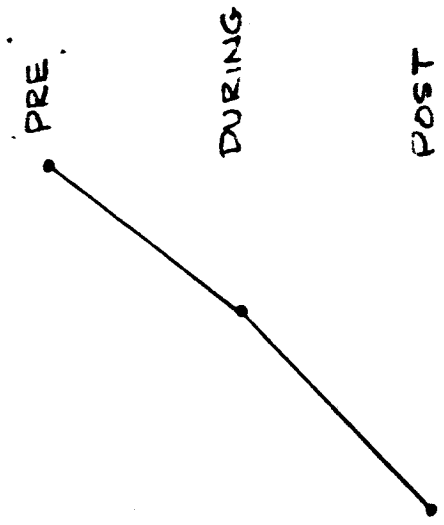

PRE
DURING
POST
PO_2
NH_3
PCO_2
Ca
TO 562
HCO_3
PROT.
UREA
GLUC.


PRE
DURING
POST
FO_2
PRE
NH_3

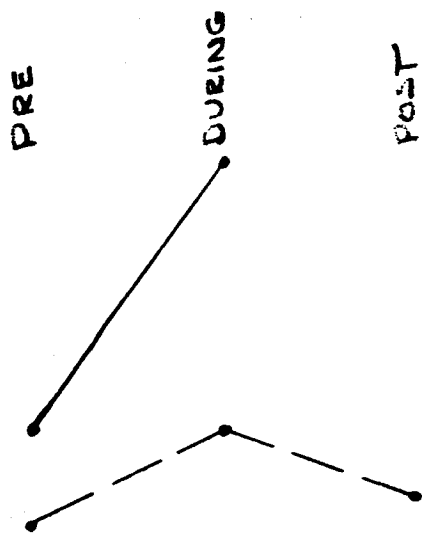

PCO_2
Calcium


PROT.

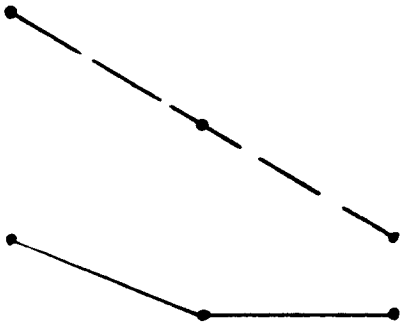

UREA
GLUC.



PO_2

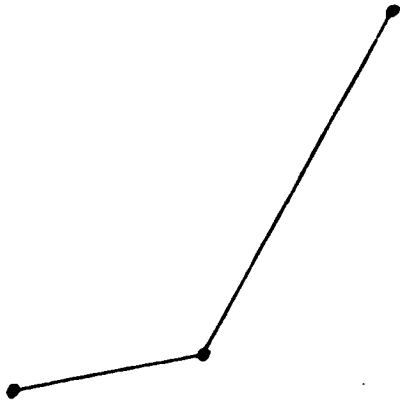


NH_3

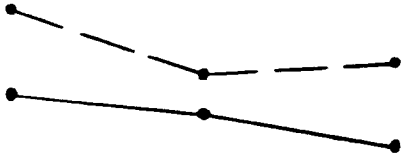


PCO_2

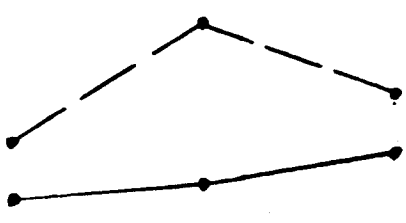
Ca.



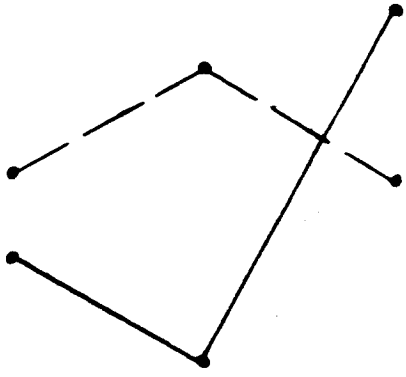
PROT.



HCO_3



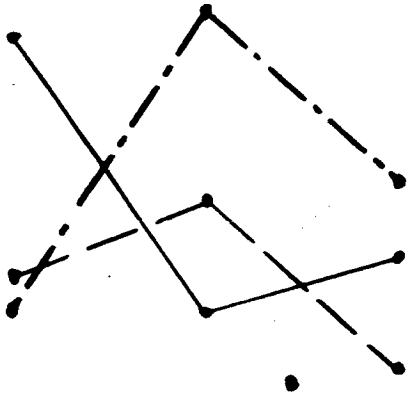
UREA



GLUC.

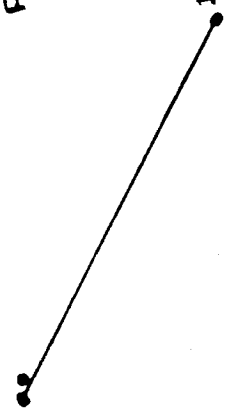
PRE DURING POST

PO_2

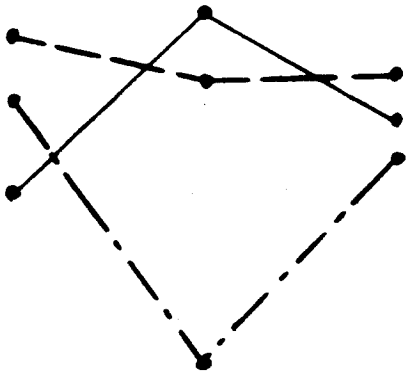


PRE DURING POST

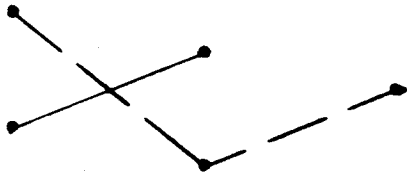
NH_3



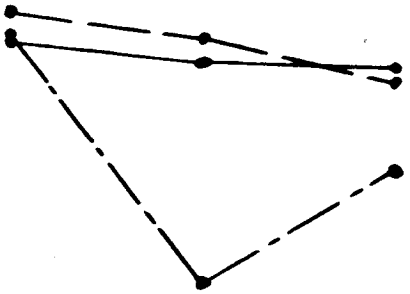
PCO_2



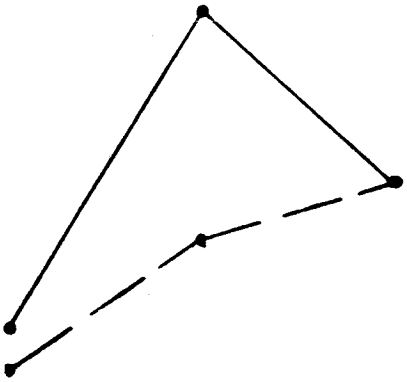
Ca



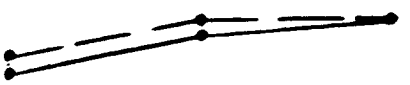
HCO_3



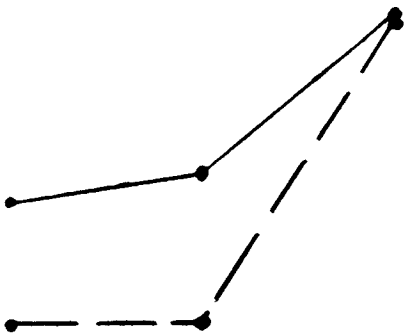
PROT.

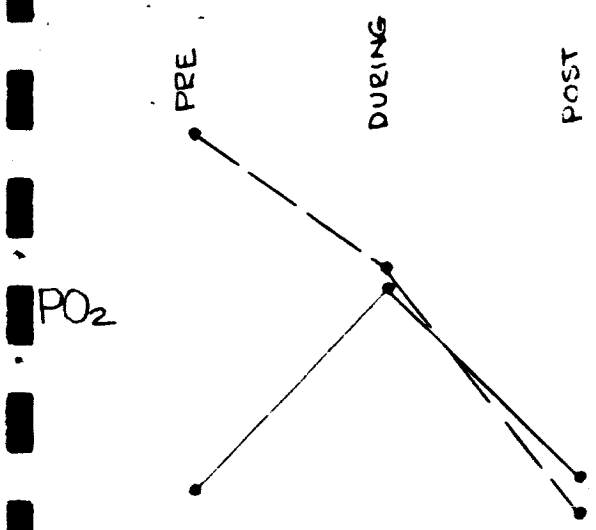


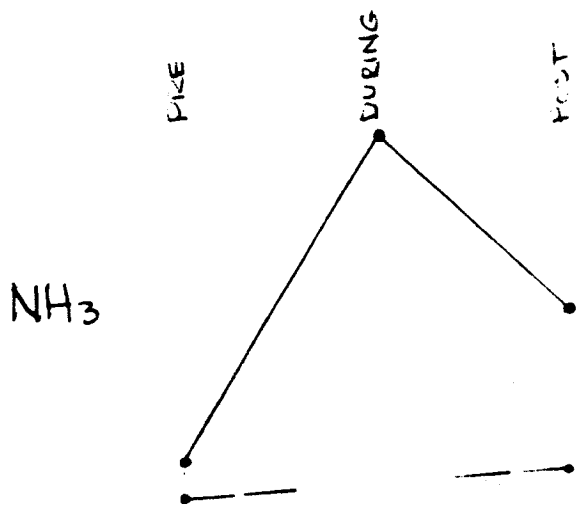
UREA

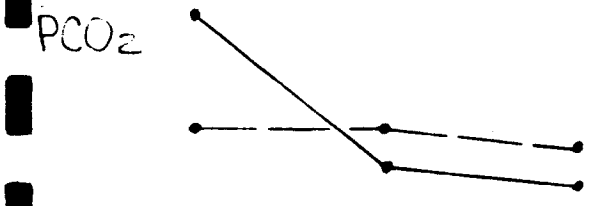


GLUC.




PRE
DURING
POST
PO_2


DURING
NH_3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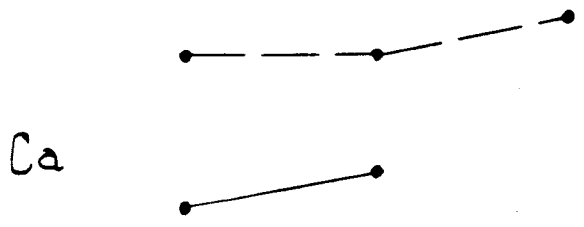
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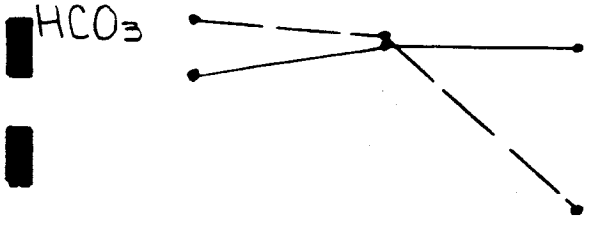
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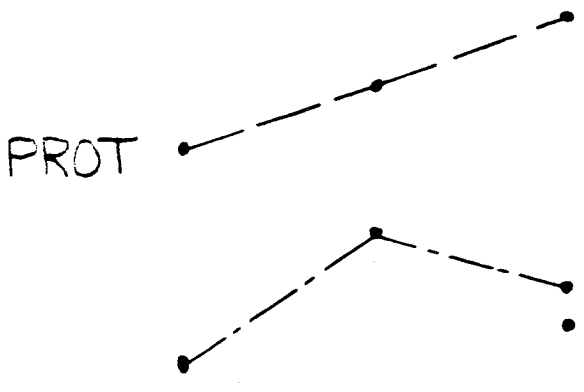
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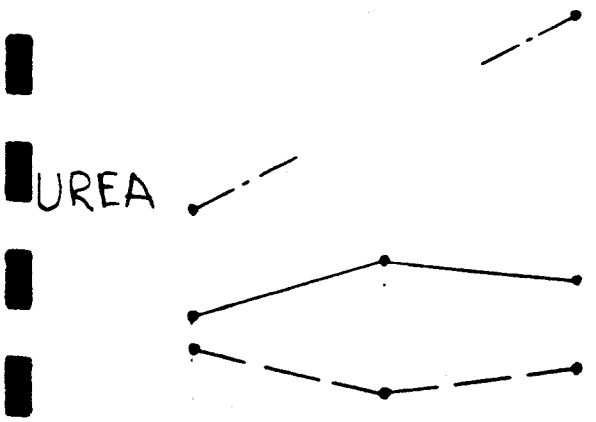
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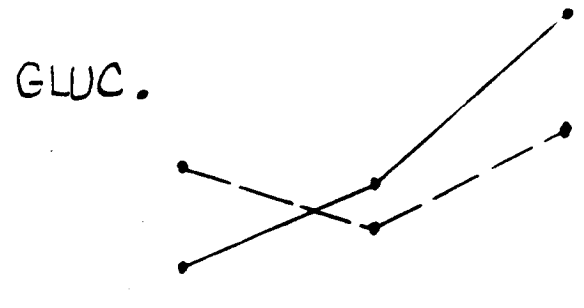
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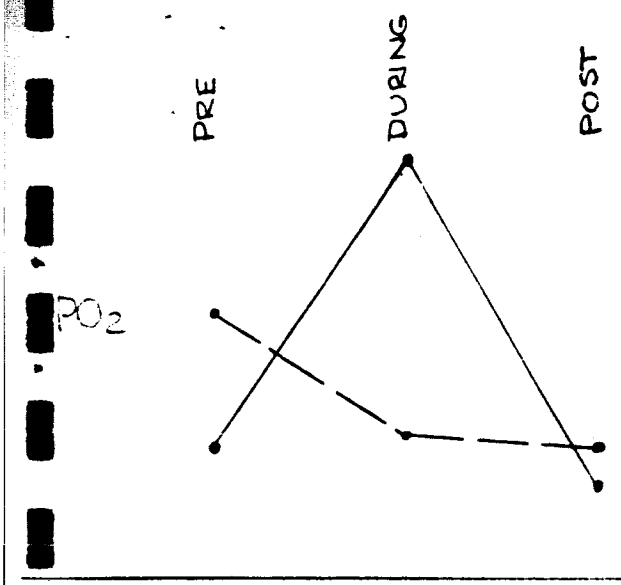
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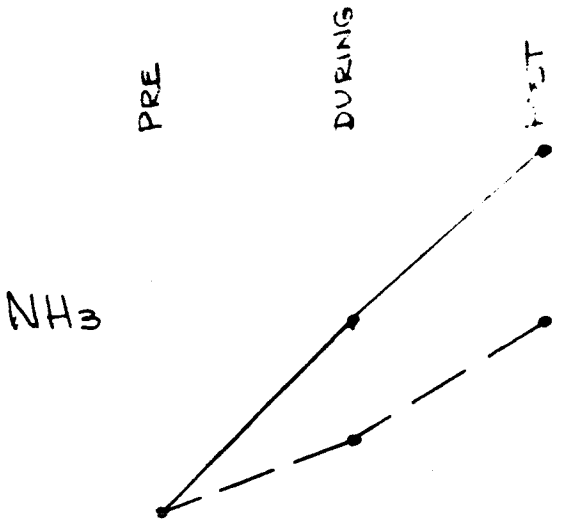
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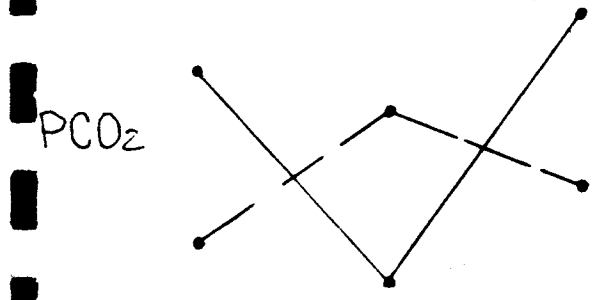
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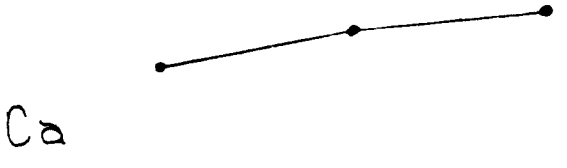
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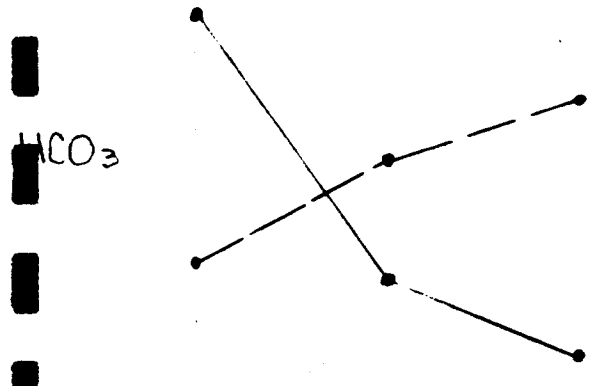
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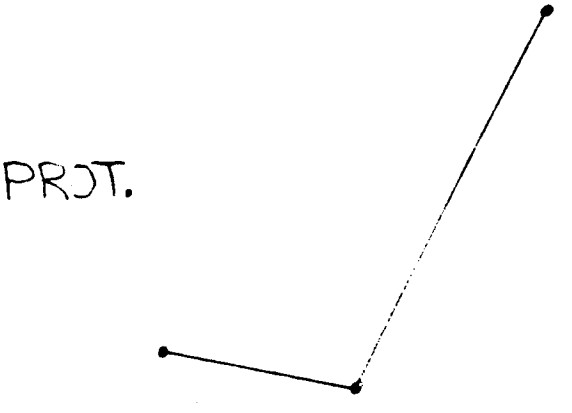
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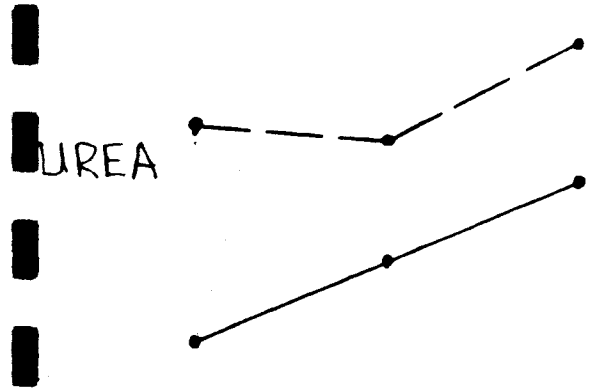
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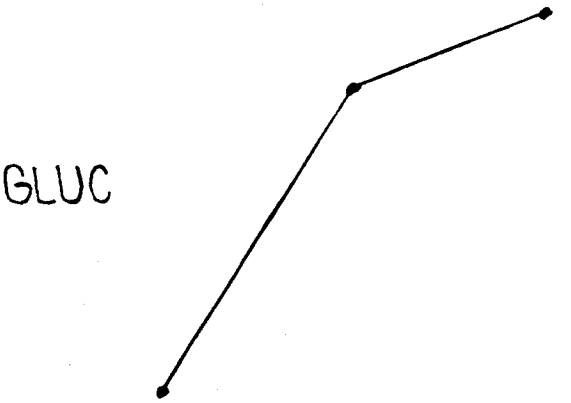
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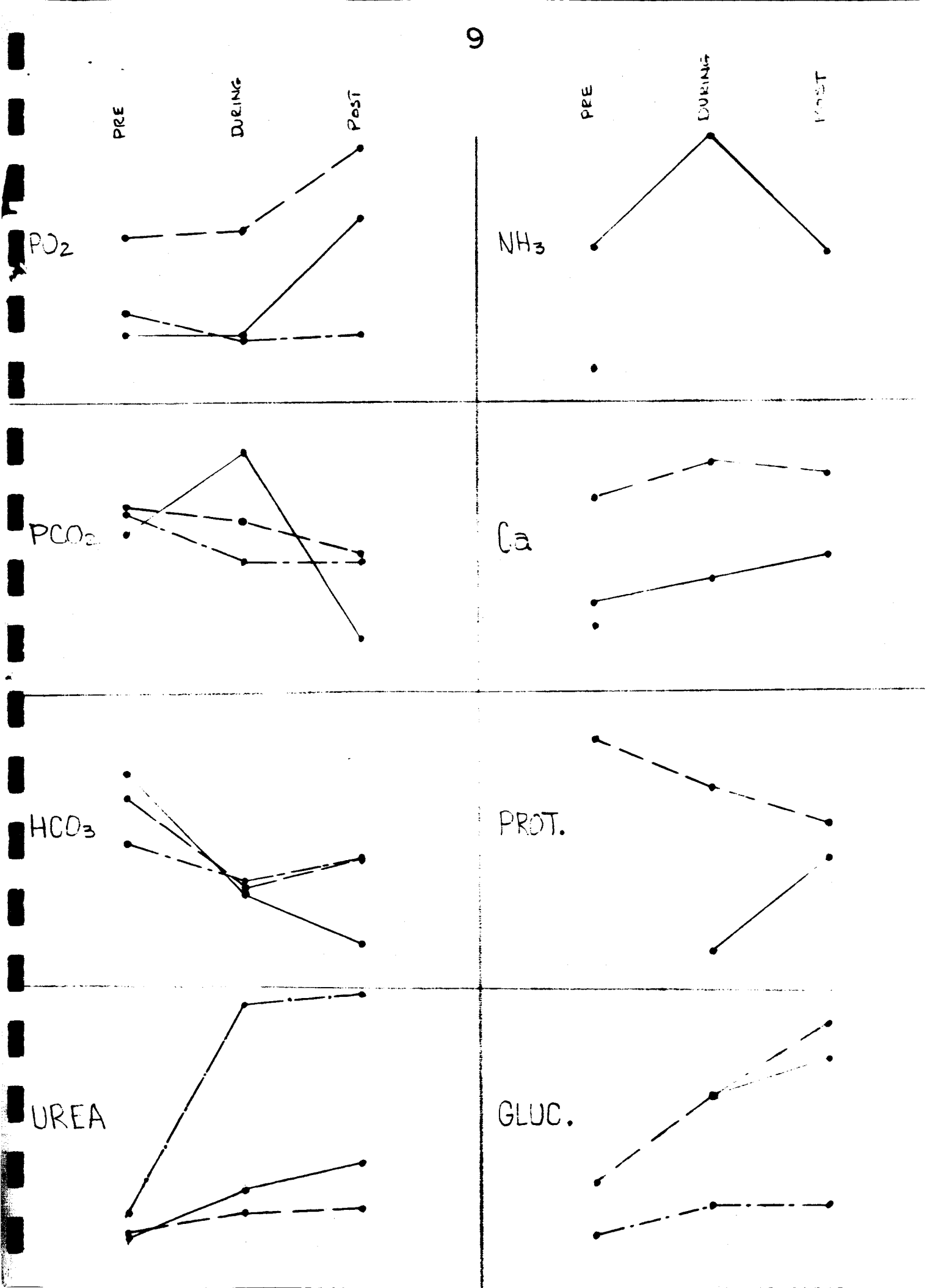
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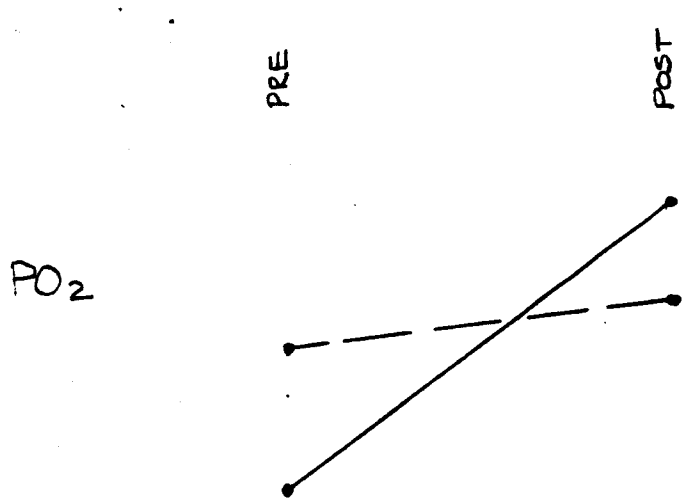
APPENDIX E

Graphs showing the variation of monitored parameters for each individual in the control group with repeated testing. Blood values shown.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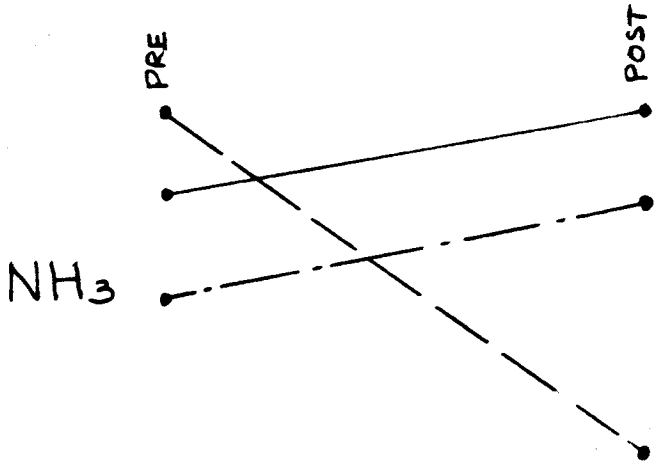
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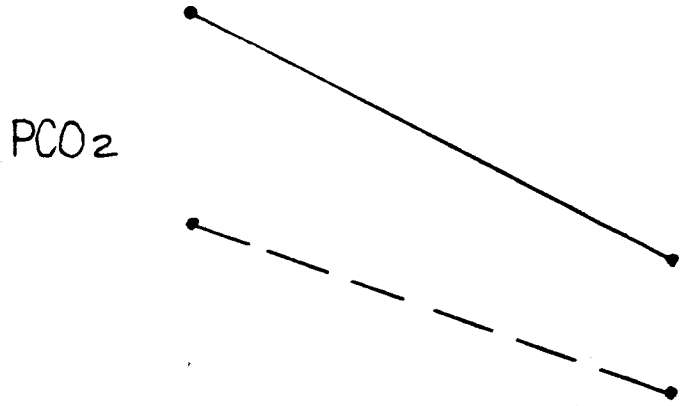
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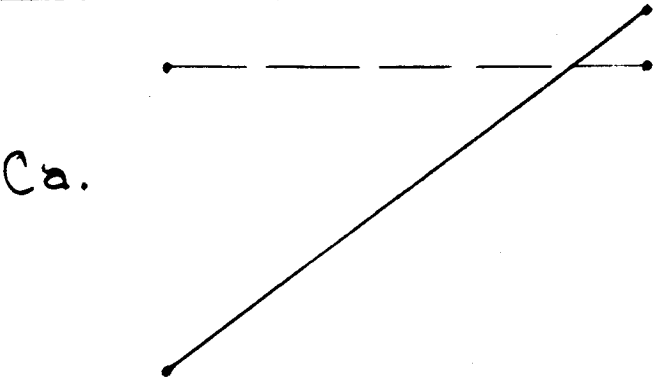
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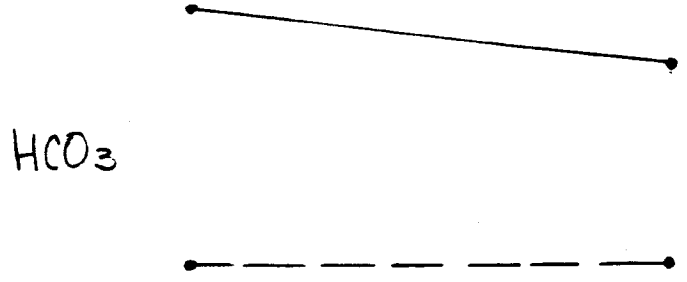
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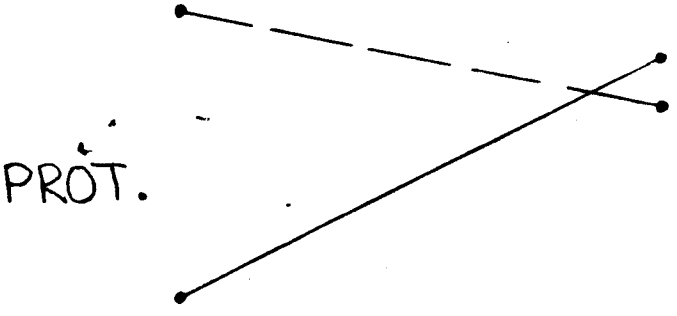
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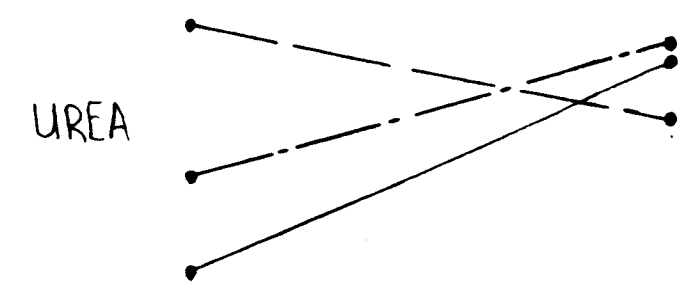
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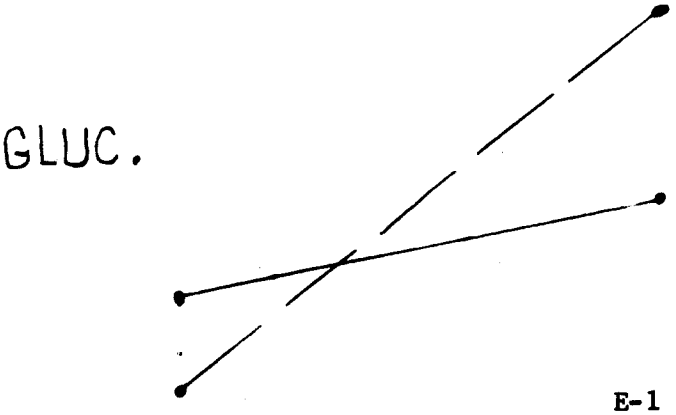
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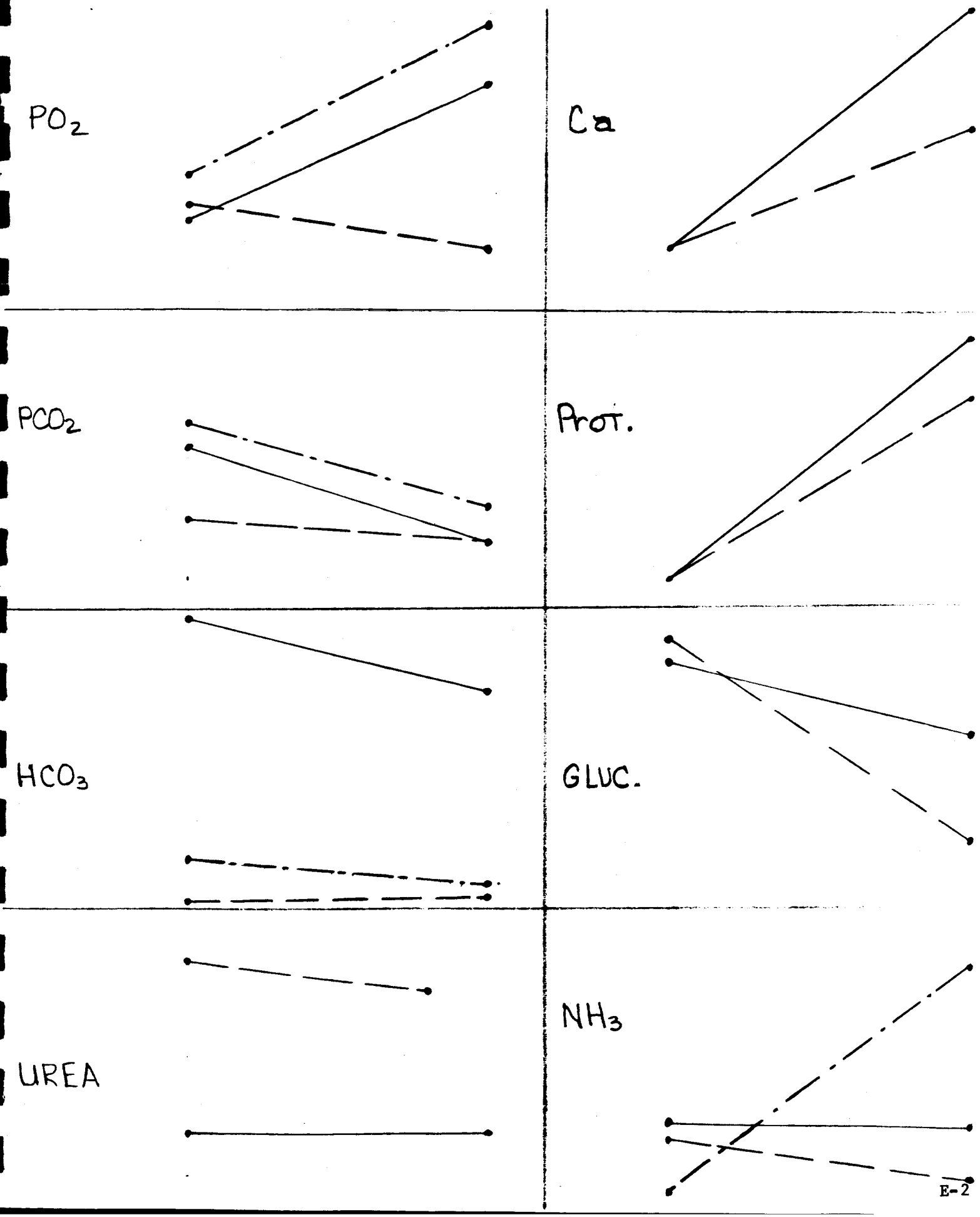
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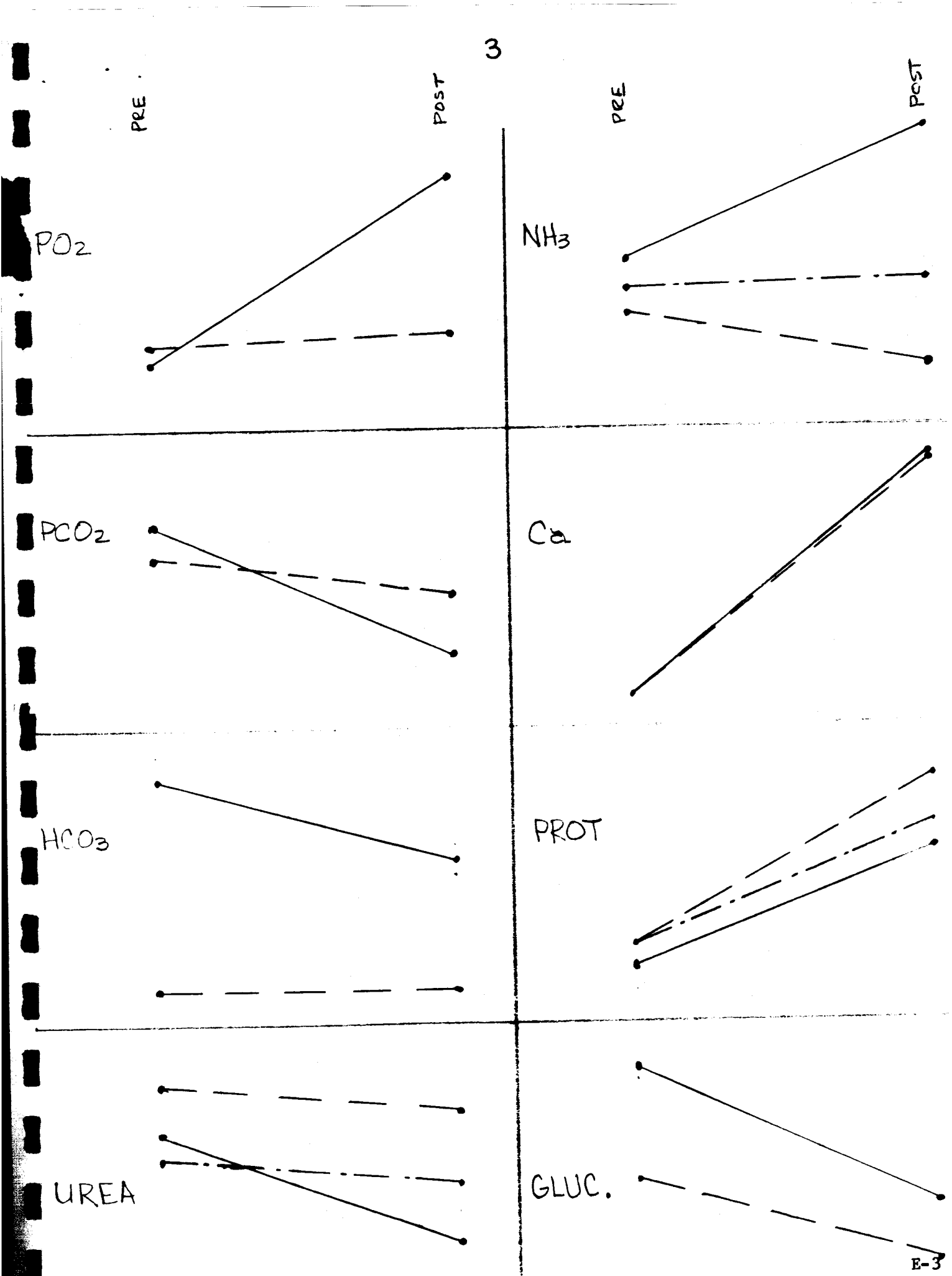
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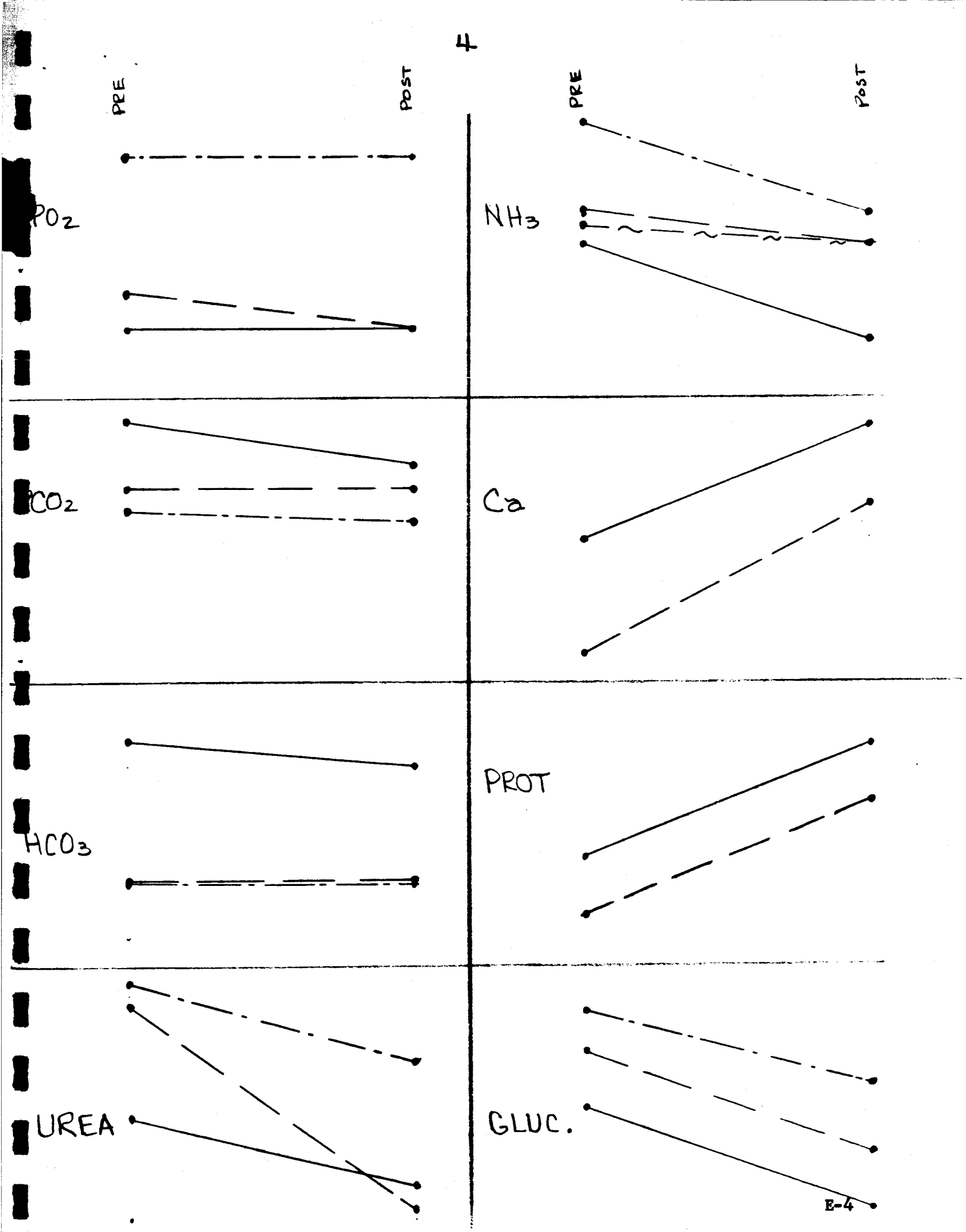
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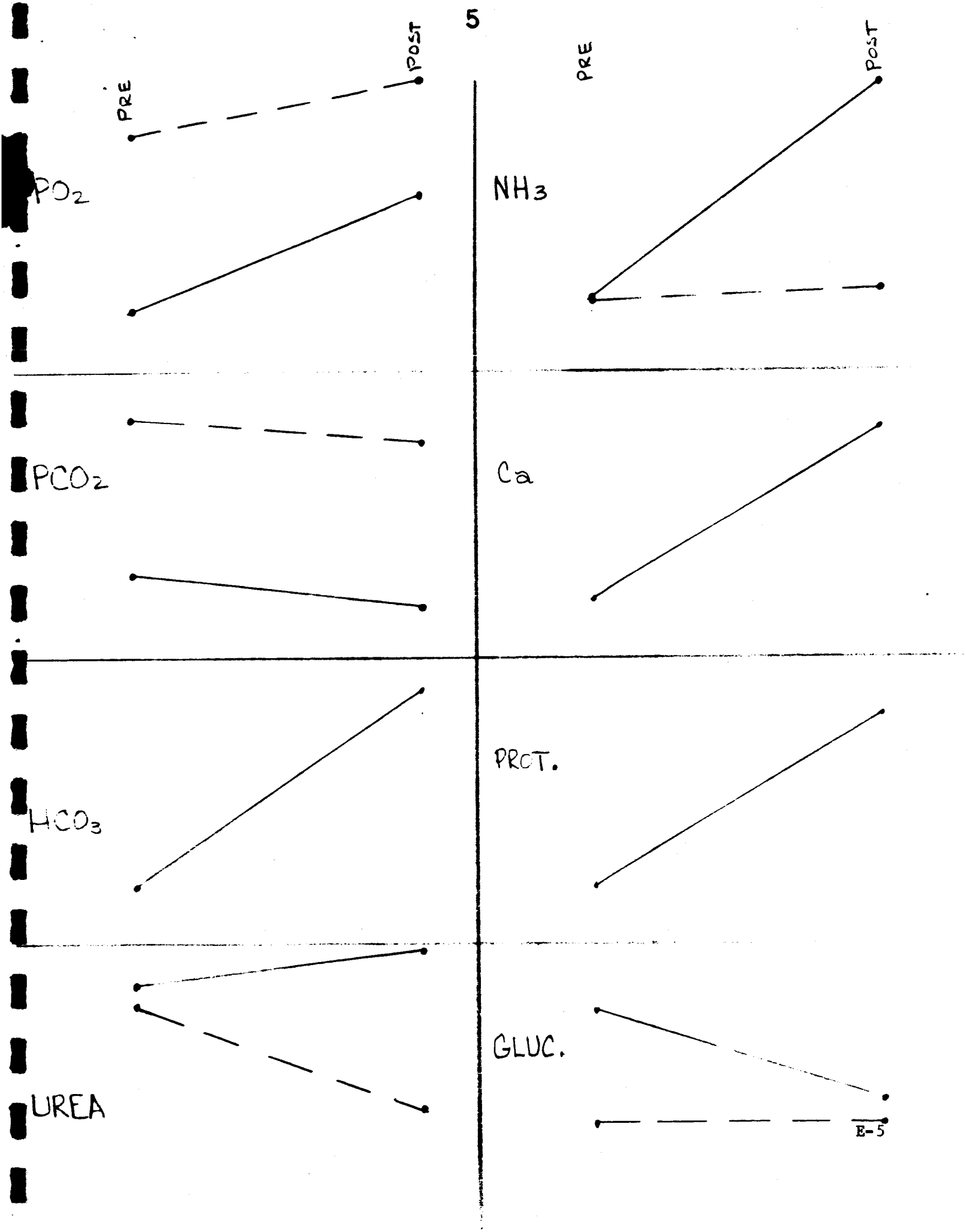
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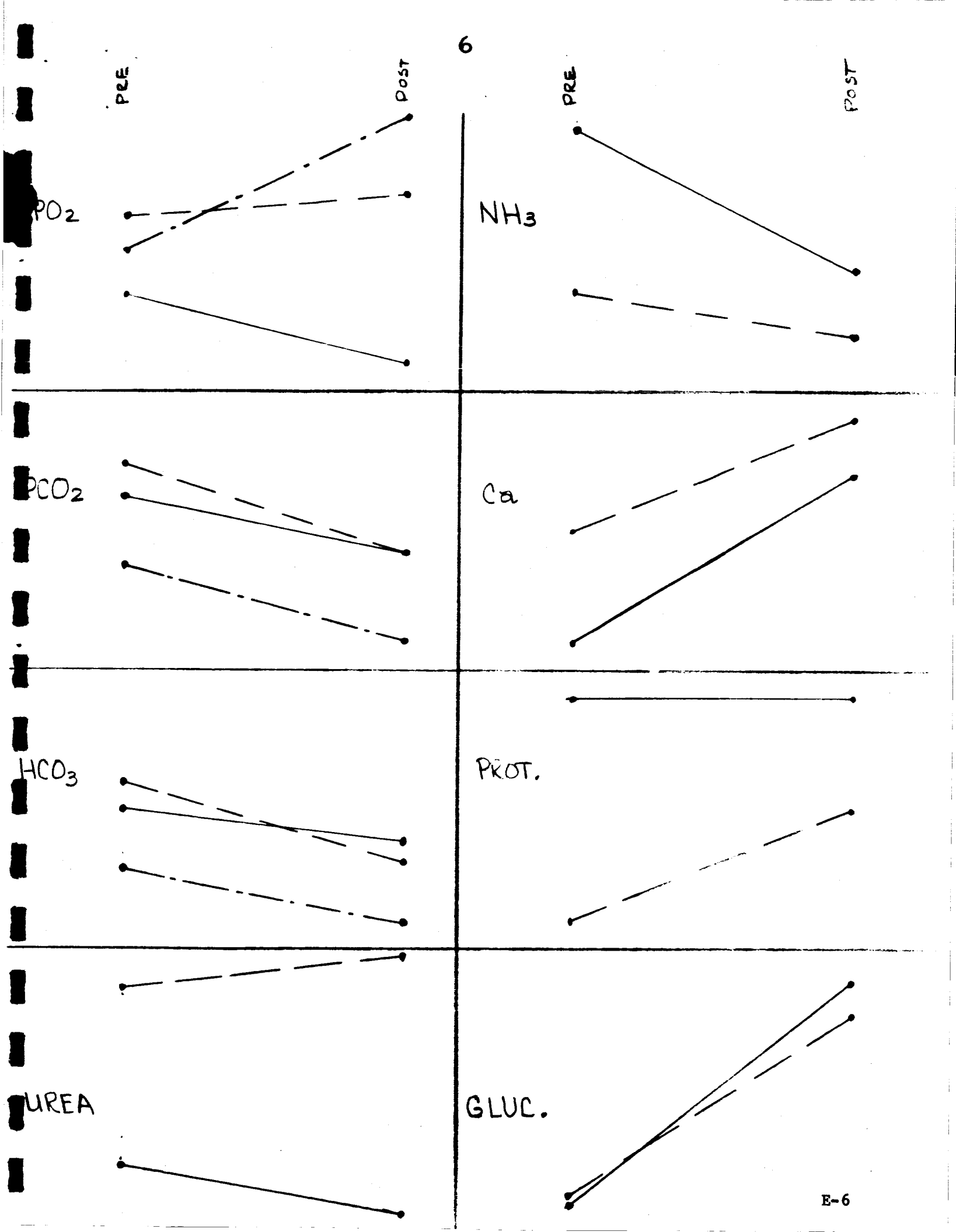
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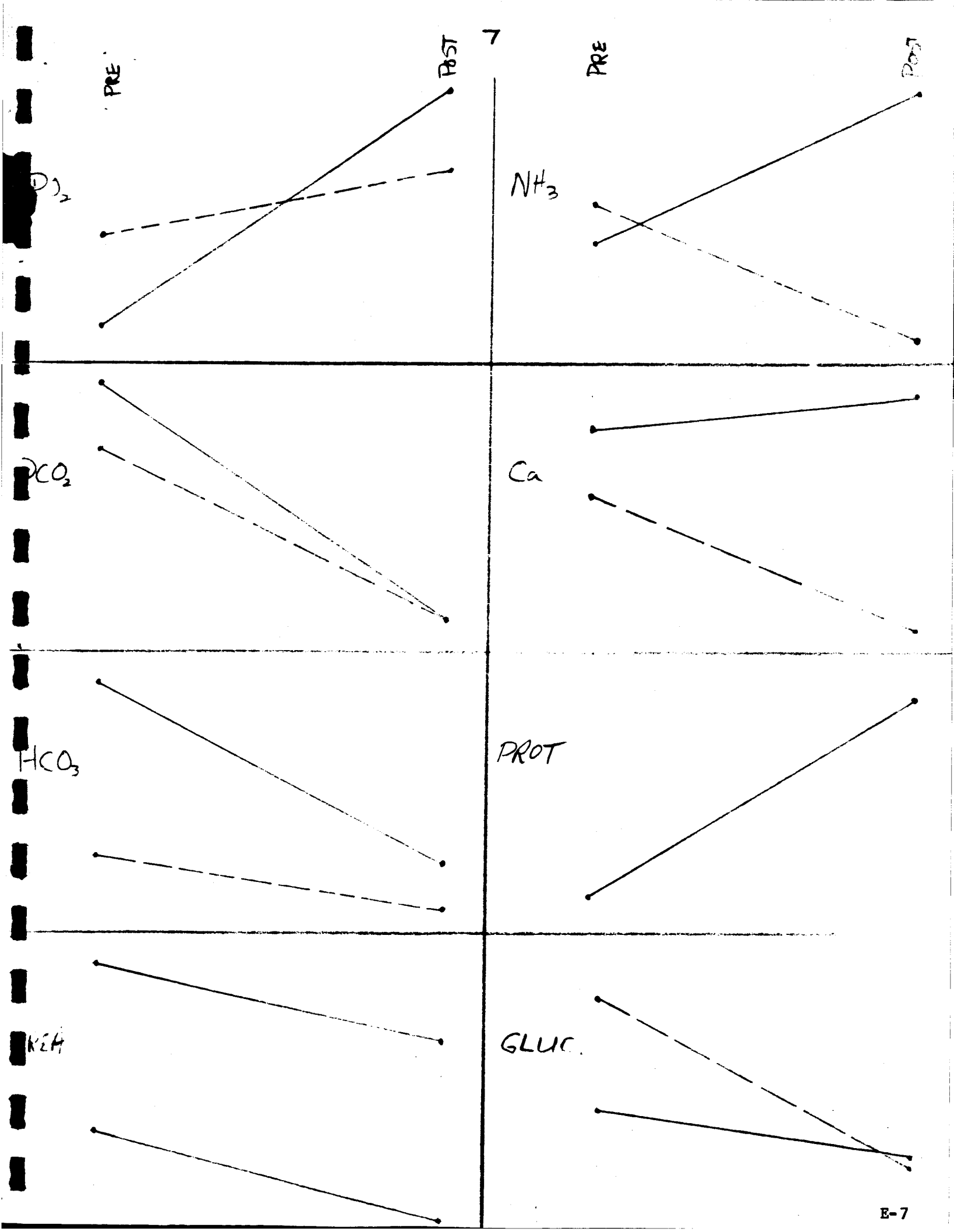
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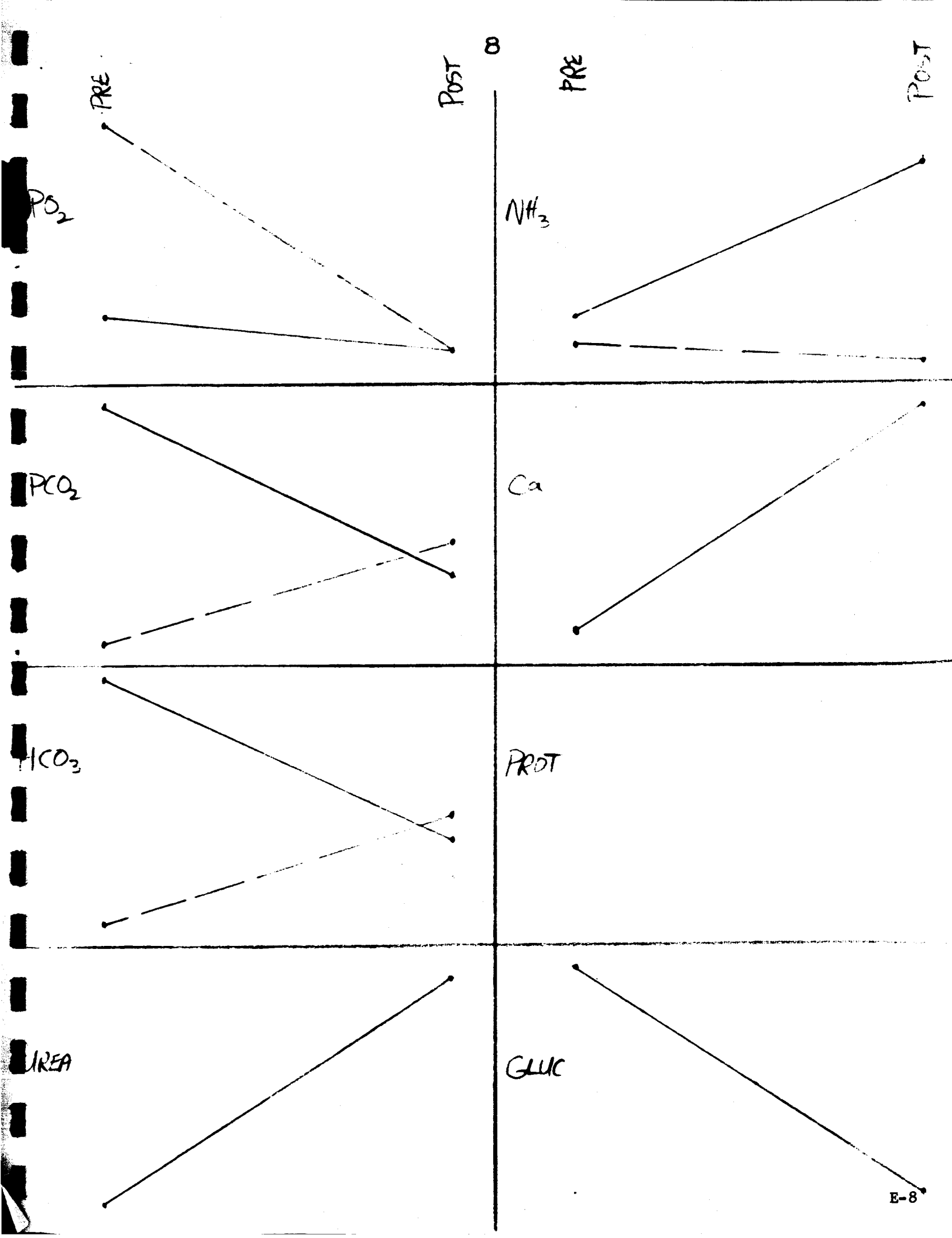
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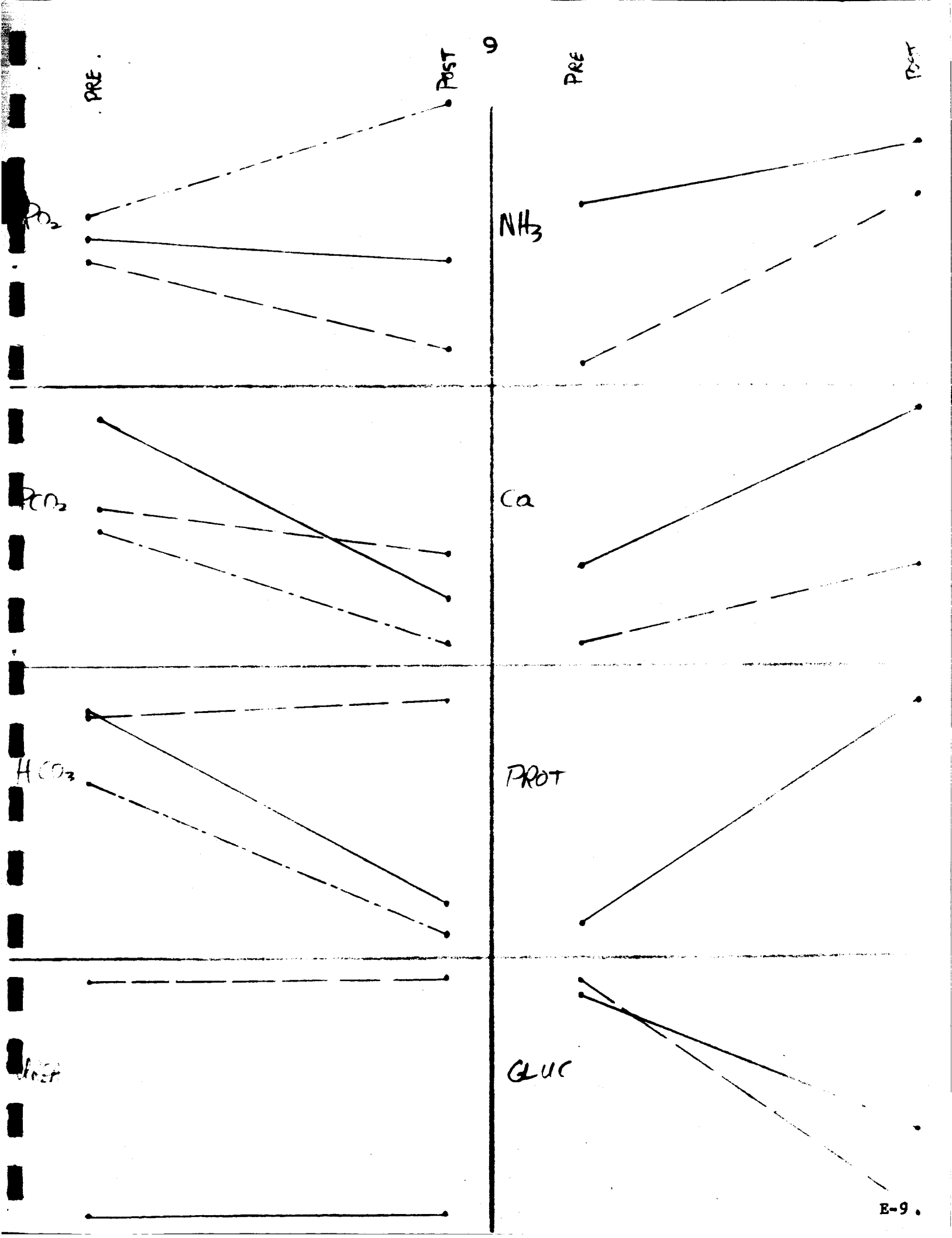
PRE
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APPENDIX F

BODY FLUID COLLECTION TECHNIQUE

APPENDIX F

Body Fluid Collection Technique

AREA I - PHYSICAL FACILITIES

Arrangements were made with two large hospitals in the Los Angeles area for appropriate space in which our technicians could obtain body fluid specimens. The first of these was Harbor General Hospital (a branch of the Los Angeles County Hospital System), which has a 600 bed rating and is located in Torrance, California. The sampling area was in the Recovery Room of the Urology Clinic Unit. The room is fifteen by thirty feet and contains ample working area, refrigeration equipment, comfortable seating and a quiet, restful environment.

The second facility was established at Orange County Hospital which has a 700 bed rating and is located in Orange, California. The Head and Neck Treatment Room containing ample working area, dental chair seating, excellent lighting and a quiet, restful atmosphere was made available for our collections.

At various times during the project it was necessary to use our own laboratory facilities at Fullerton to sample in-house personnel associated with the study and select volunteers. Also, at times it was difficult for subjects to report to a central sampling location within the hospital, thus necessitating setting up of a remote sampling depot to handle personnel from a particular unit.

The control group study was initiated and maintained at the El Toro Base Hospital where we received full use of the Solarium area for our sampling.

At all times, whether sampling at a central hospital location, a designated unit within the hospitals, or our own laboratory, the following conditions were met:

1. Subject comfort
2. Freedom from distraction and disturbances
3. Non-stressful environment

AREA II - DISCUSSION OF POPULATION GROUPS

1. "Control" Group Description - Nine officers at the U. S. Marine Corps Air Station, El Toro, volunteered to participate in the parotid fluid study program. Except for one subject, all men have pilot assignments ranging from reconnaissance to high performance aircraft. Their ages were from 26 to 35. None of the subjects were under medical treatment except subject #2 who was taking appetite depressant tablets. Subject #2 also was treated for mononucleosis in April 1961. This was the only illness requiring significant medical attention in the past five years.

2. "Normal" Group Description - The normal population totaled 34 subjects of which 18 were males and 16 were females. None of the subjects were hospitalized nor was there knowledge of their undergoing significant medical attention at the time of sampling.

3. "Hospital" Group Description - The hospital population totaled 59 subjects of which 50 were males and 9 were females. All were confined on an in-patient basis and were undergoing significant medical attention at the time of sampling. A representative listing of the diseased states is listed below:

- o Rectal Tumor
- o Senile Emphysema
- o Hepatitis
- o Trans Urethral Prostatectomy
- o Fractured Pelvis
- o Pneumonia
- o Obstructive Jaundice
- o Nephritis
- o Cellulitis
- o Appendectomy

AREA III - SAMPLING EQUIPMENT

1. Parotid - Our parotid fluid collector is a spring tension apparatus that utilizes mechanical pressure rather than vacuum in order to keep the cup securely in place over Stensen's duct. At the same time, the pressure is not strong enough to restrict flow from the orifice. Bilateral sampling is easily achieved with two of the devices. Both the cup and cheek plate are machined from Delrin and the entire collector can be autoclaved.

2. Blood - The required blood samples were drawn using the Vacutainer system. This allowed flexibility insofar as volume and choice of anticoagulants (if needed) were concerned. All venipunctures were performed by skilled medical technologists, thus affording a minimum of discomfort to the subject. All tubes were promptly labeled with subject's name and date immediately after phlebotomy.

3. Urine - A 2500 ml capacity plastic bag was used for collection of all 24-hour urine specimens.

4. Stimulant - The oral stimulant used to promote flow were sour crystalline pieces marketed under the tradename of "Regal Lemon Sours." Stimulation was required in order to provide sufficient sample volume for analysis of the required components and keep the sampling time to approximately 15 minutes maximum.

AREA IV - SAMPLING PROCEDURE

Perhaps the most important part of the sampling protocol was that of placing the subject at ease. This was accomplished by a brief explanation of equipment, why we were conducting these tests (to find a better means of evaluating an individual biochemically), and what the program hoped to accomplish (to find correlations between parotid, blood, and urine). It was noted that subjects prepared in this manner were much more relaxed and willing to cooperate. Listed below are the stepwise procedures that were followed in performing the body fluid collections on normal and hospitalized populations.

1. Parotid Collection

a. The subject was seated comfortably (if ambulatory) and rapport was established as previously described.

b. Gathering of personal data on laboratory sheets.

c. The technician scrubbed his hands thoroughly.

d. Preliminary oral examination. This was necessary due to the anatomical variation among people as to the size and location of Stensen's duct. It was not a requirement for subjects to remove dentures (if worn) in order to affix the parotid cups.

e. Insertion of collection cups.

f. Stimulation was initiated by placing a sour lemon drop on the tongue. Parotid flow was followed by visual observation down the collecting tube. When the first drop entered the pre-weighed collection tube, a stopwatch was started. The first minute of flow was considered a washing or flushing of the gland and subsequently discarded. From one to four samples were collected at known time increments and were labeled as to subject name, date, and time increment immediately upon completion. Later, the tubes containing the parotid fluid were re-weighed, the tare subtracted and flow rates (as H_2O) were calculated on the basis of milliliters per minute.

g. A pulse rate was taken during the collection period.

h. Parotid collectors were removed and placed in a 70% alcohol bath.

2. Blood Collection

a. Upon completion of parotid fluid collection, the subject's arm was prepared for venipuncture. A tourniquet was applied and the anticubital area of the arm was thoroughly cleansed.

b. Venipuncture was performed and blood drawn into a predetermined number and type of tube.

c. A bandage was applied to the site and a few minutes allowed for subject recovery.

d. Blood tubes were promptly labeled with subject name and date.

3. Urine Collection

a. Oral instructions were given at the same time the collection bag was identified by subject name and date. This served to reinforce the directions already on the bag as part of the label. Subjects were asked to return bags to the central collection area from where our technicians could transport them back to the Fullerton laboratory facilities.

AREA V - CONTROL GROUP SAMPLING PROCEDURE

From each subject, a series of two urine, two blood, and five parotid samples were obtained. Sampling was undertaken approximately two hours after eating. An initial urine, parotid, and blood sample was taken. The subject then commenced a body function routine which consisted of three 10 minute exercise cycles with concomitant parotid sampling. Following the exercise, a final parotid sample was collected during a 15 minute recovery period. Subsequently the second blood and urine samples were taken.

Appendix G

ANALYTICAL PROCEDURES

1. UREA NITROGEN

1.1 REFERENCE

Chaney, A.L., and E.P. Marbach, Clin. Chem. 8, 131 (1962).

1.2 REAGENTS

1.2.1 Buffered Urease Solution. 150 mg. urease (ca. 1000 U/g.) and 1.0 g. ethylene-diaminetetraacetic acid per 100 ml. aqueous solution, adjusted to a pH of 6.5. The urease solution is stable for 1 month in the refrigerator. Stability is much greater in the frozen state.

1.2.2 Phenol Color Reagent. 50.0 g. phenol and 0.25 g. sodium nitroprusside per liter. Stable at least 2 months if kept cool and in amber bottle protected from light.

1.2.3 Alkali-Hypochlorite Reagent. 25.0 g. NaOH and 40 ml. Clorox per liter. Stable at least 2 months if kept cool and in amber bottle protected from light.

1.2.4 Urea Standard. Place 0.2145 g. urea in a 1 l. volumetric flask and make to volume with distilled water. Add a few drops of chloroform as a preservative. Store in refrigerator. 1 ml. contains 0.1 mg. urea N.

1.3 PROCEDURE

1.3.1 Set up the following in test tubes:

Blank. 0.2 ml. buffered urease solution.

Standard. 0.2 ml. buffered urease solution and 20 ul. urea standard.

Unknown. 0.2 ml. buffered urease solution and 20 ul. serum or parotid fluid.

1.3.2 Incubate tubes in a water bath at 37°C. for 15 min. or at 25°C. for 30 min. (these are minimum times).

1.3.3 Add 1.0 ml. phenol color reagent to each tube, mix, then add 1.0 ml. alkali-hypochlorite reagent and mix promptly again.

1.3.4 Incubate tubes at 37°C. for 20 min., or at 25°C. for 40 min.

1.3.5 Add 8 ml. water to each tube. Mix by inversion using clean Saran Wrap or Parafilm "M" over top of tube.

1.3.6 Read absorbances of blank (A_b), standard (A_s), and unknown (A_x) against water at 630 mu.

1.4 CALCULATION

$$\text{mg. urea N/100 ml.} = \frac{A_x - A_b}{A_s - A_b} \times 10$$

2. CALCIUM, PAROTID FLUID AND SERUM

2.1 REFERENCE

Bachra, B. N., Dauer, A., and A. E. Sobel, Clin. Chem. 4, 107, (1958).

2.2 REAGENTS

2.2.1 KOH, 1.25 N. Dissolve 70 g. KOH pellet in 500 ml. of water and add water to 1 liter.

2.2.2 EDTA Solution. Dissolv e 395 mg. disodium ethylenediamine-tetraacetate dihydrate in 500 ml. water and add water to 1 liter.

2.2.3 Cal-Red Indicator Solution. Grind 1.0 g. "Cal-Red Dilute" in 10 ml. water. Not all the material goes into solution. This suspension is usually stable in the refrigerator for about two weeks.

2.2.4 Caprylic Alcohol.

2.2.5 Calcium Standard. Weigh out exactly 250 mg. Iceland Spar and transfer to a 1 liter volumetric flask. Add 7 ml. dilute HCl (1 part conc. HCl + 9 parts water). Let stand until solid is dissolved, then add approximately 900 ml. water. Adjust pH to 6.0 with 50% ammonium acetate. Adjust volume to 1 liter with water and mix. 1 ml = 100 ug Ca.

2.3 PROCEDURE

2.3.1 Add the following to clear, conical-tipped 12 or 15 ml. centrifuge tubes:

Standard. 0.50 ml. (50 ug Ca).

Unknown. 0.50 ml. serum or parotid fluid and a very small drop of caprylic alcohol.

2.3.2 Proceed with 1 tube at a time (if samples remain alkaline for more than 10 min., the end point is not sharp). Add 2.5 ml. 1.25 N KOH, mix, and add 0.2 ml. indicator solution (shake indicator solution well each time before pipetting).

2.3.3 Immediately titrate with EDTA solution until color changes from wine-red to blue. Observe the color change by looking through the solution against an incandescent light.

2.4 CALCULATION

$$\text{meq. Ca/l.} = \frac{\text{ml. unknown titration}}{\text{ml. standard titration}} \times 5$$

3. CALCIUM, URINE

3.1 REFERENCE

Bachra, B. N., Dauer, A., and A. E. Sobel, Clin. Chem. 4, 107, (1958).

3.2 REAGENTS

3.2.1 KOH, 1.25 N. See procedure 2.2.1.

3.2.2 EDTA Solution. See procedure 2.2.2.

3.2.3 Cal-Red Indicator Solution. See procedure 2.2.3.

3.2.4 Sodium Citrate, 0.05 M. Dissolve 14.7 g. of the dihydrate per liter water.

3.2.5 Ammonium Oxalate, 10%.

3.2.6 NH_4OH, 2%.

3.2.7 HCl, 1 N.

3.2.8 Calcium Standard. See procedure 2.2.4.

3.3 PROCEDURE

3.3.1 If urine is clear and no precipitate is seen clinging to walls of container, mix and remove approximately 10 ml. Acidify to pH 1 with conc. HCl. If precipitate is present in original urine, acidify the entire urine specimen to pH 1.

3.3.2 Warm acidified specimen to 60°C for at least 15 min., mixing occasionally.

3.3.3 Pipet 0.50 ml. urine from step 2 into a conical-tipped, 12 or 15 ml. centrifuge tube, add 0.1 ml. 10% ammonium oxalate, and mix. Add 1 drop of methyl red indicator and then 2% NH_4OH dropwise to obtain orange color.

3.3.4 Place in boiling water bath for 20 min. Cool to room temperature, centrifuge, decant supernatant fluid carefully, invert and drain on absorbent paper.

3.3.5 Dissolve precipitate in 0.25 ml. 1 N HCl with warming and add 0.25 ml. 0.05 M sodium citrate.

3.3.6 To a clear, conical-tipped centrifuge tube, pipet 0.5 ml. of standard (50 ug Ca).

3.3.7 Proceed with 1 tube at a time (if samples remain alkaline for more than 10 min., the end point is not sharp). Add 2.5 ml. 1.25 N KOH, mix, and add 0.20 ml. indicator solution (shake indicator solution well each time before pipetting).

3.3.8 Immediately titrate with EDTA solution until color changes from wine-red to blue. Observe the color change by looking through the solution against an incandescent light.

3.4 CALCULATION

$$\text{meq. Ca/l. urine} = \frac{\text{ml. unknown titration}}{\text{ml. standard titration}} \times 5$$

4. TOTAL PROTEIN, SERUM (BIURET REACTION)

4.1 REFERENCE

Henry, R.J., Sobel, C., and S. Berkman, Anal. Chem. 29, 1491 (1957).

4.2 REAGENTS

4.2.1 Biuret Reagent. (a) Dissolve 17.3 g. $CuSO_4 \cdot 5H_2O$ in about 100 ml. hot water. (b) Dissolve 173 g. sodium citrate and 100 g. anhydrous Na_2CO_3 in about 800 ml. water by heating. When cool, pour (a) into (b) while stirring and dilute to 1 l. at room temperature. Stable indefinitely at room temperature.

4.2.2 NaOH, 3%.

4.3 PROCEDURE

4.3.1 Set up in test tubes:

Reagent Blank. 5 ml. 3% NaOH.

Sample. Rinse 0.1 ml. serum from a 0.1 ml. TC pipet into 4.9 ml. of 3% NaOH.

4.3.2 Add 1 ml. biuret reagent to each tube and mix. This step should follow step 1 without delay.

4.3.3 Wait at least 15 min., examine for Tyndall effect. If sample is significantly turbid, add about 3 ml. of ether, shake vigorously for 30 sec., and centrifuge.

4.3.4 Read absorbance of sample against reagent blank at 545 mu.

4.4 CALCULATION

The $A_{1\ cm.}^{1\%}$ at 545 mu and 24°C., reading against a reagent blank, is 3.27.

g. protein/100 ml. = A_x x 18.35

5. TOTAL PROTEIN, PAROTID FLUID (BIURET REACTION)

5.1 REFERENCE

Henry, R.J., Sobel, C., and S. Berkman, Anal. Chem. 29, 1491 (1957).

5.2 REAGENTS

5.2.1 Biuret Reagent. See procedure 4.2.1.

5.2.2 NaOH, 6%.

5.3 PROCEDURE

5.3.1 Set up in test tubes:

Reagent Blank. 1.0 ml. water.

Sample. 0.5 ml. parotid fluid and 0.5 ml. water.

5.3.2 To each, add 1.0 ml. 6% NaOH and 0.4 ml. biuret reagent.

5.3.3 Wait at least 15 min., examine for Tyndall effect. If sample is significantly turbid, add about 3 ml. of ether, shake vigorously for 30 sec., and centrifuge.

5.3.4 Read absorbances of samples against reagent blank at 545 mu.

5.4 CALCULATION

$$\text{mg. protein/100 ml.} = 1468\ A$$

6. GLUCOSE

6.1 REFERENCE

Cawley, L.P., Spear, F.E., and R. Kendall, Am. J. Clin. Path. 32, 195 (1959).

Worthington Biochemical Corporation Instruction Manual.

6.2 REAGENTS

6.2.1 Zinc sulfate, 10%. 100 g. $ZnSO_4 \cdot 7H_2O$/liter.

6.2.2 NaOH, 0.5N. 20 g./liter.

6.2.3 Glucostat Reagent (Worthington Biochemical Corp., Freehold, New Jersey). The reagents are supplied dry in a set of 2 vial. Contents of the small vial (chromogen: O-dianisidine) are dissolved in 1 ml. of methyl alcohol and added to a 100 ml. volumetric flask containing 50 ml. of distilled water. The contents of the large vial (glucostat reagent: glucose oxidase, horseradish peroxidase, and phosphate buffer) are dissolved in distilled water and added to the volumetric flask, which is then diluted to volume. The reagent may be stored in a brown bottle at -5°C. for several days without loss of activity.

6.2.4 HCl, 5N.

6.2.5 Stock Glucose Standard, 1%. Use highest purity glucose. Saturate with benzoic acid. Stable indefinitely in refrigerator. 1 ml. contains 10 mg. glucose.

6.2.6 Dilute Standard (a). Dilute 1 ml. of stock standard to 500 ml. with water. 1 ml. contains 0.02 mg. glucose.

6.2.7 Dilute Standard (b). Dilute 1 ml. of stock standard to 200 ml. with water. 1 ml. contains 0.05 mg. glucose.

6.3 **PREPARATION OF FILTRATES.**

6.3.1 Plasma - Add 0.5 ml. plasma to 8.5 ml. H_2O and mix. Add 0.5 ml. zinc sulfate, mix, add 0.5 ml. 0.5N NaOH, and mix again. Let stand 5 min. and filter.

6.3.2 Parotid Fluid - Add 0.5 ml. parotid fluid to 1.5 ml. H_2O and mix. Add 0.25 ml. zinc sulfate, mix, add 0.25 ml. 0.5 N NaOH, and mix again. Let stand 5 min. and filter.

6.3.3 Urine - Neutralize the urine with the least amount of acid or alkali. If appreciable amounts are required, the dilution should be taken into account. It is convenient to make a preliminary qualitative estimation with one of the paper strip tests. Then dilute the urine to contain from approximately 0.1 to 0.3 mg. of glucose per ml. to 10 ml. of urine, add ca. 0.5 g. of activated charcoal. Stir and let stand 15 min., then filter clear.

6.4 **PROCEDURE**

6.4.1 Set up in Test Tubes:

Reagent Blank. 0.5 ml. H_2O.

Standard (1). 0.5 ml. dilute standard (a).

Standard (2). 0.5 ml. dilute standard (b).

Sample. 0.5 ml. filtrate.

6.4.2 At 15 second intervals, add 1.5 ml. of glucostat reagent to each tube.

6.4.3 After exactly 30 min. for each tube, add 0.05 ml. 5N HCl and mix.

6.4.4 Read absorbances of blank (A_b), standard (A_{s1} or A_{s2}), and sample (A_x) against water at 410 mu.

6.5 CALCULATION

<u>Plasma</u> - mg. glucose/100 ml. $= \frac{A_x - A_b}{A_{s2} - A_b} \times 0.25 \times \frac{100}{.5} \times 20$

<u>Parotid Fluid</u> - mg. glucose/100 ml. $= \frac{A_x - A_b}{A_{s1} - A_b} \times .01 \times \frac{100}{.5} \times 5$

<u>Urine</u> - mg. glucose/100 ml. $= \frac{A_x - A_b}{A_{s1} - A_b} \times .01 \times \frac{100}{.5} \times$ dilution

7. AMMONIA NITROGEN, BLOOD, AND PAROTID FLUID

7.1 REFERENCE

Seligson, D., and K. Hirahara, J. Lab. Clin. Med. 49, 962 (1957).

Henry, R.J., Clinical Chemistry: Principles and Techniques, p. 327.

7.2 APPARATUS

7.2.1 Diffusion Bottles. Vaccine bottles, ca. 50-65 ml. capacity, dimensions ca. 2.5 x 1.5 in.

7.2.2 Rubber Stoppers. One set of solid stoppers to fit the above bottles. A second set with one hole to fit the receiving rods.

7.2.3 Receiving Rods. 6 x 80 mm. glass rod with 0.5 in. of one end ground so that when dipped in acid, it retains an acid film over its surface. The rods are set in the one hole rubber stopper so that when placed in the bottle the ground end of the rod extends about half-way into the bottle.

7.2.4 Stainless Steel Rods. Approximately 8 mm. in diameter and 40 mm. long. Three are required per bottle.

7.2.5 Rotator. A wheel, rotating about 50 r.p.m. with the axis horizontal, to which spring clamps are attached to hold the diffusion bottle about 8 cm. from the axis and with the flat bottom of the bottle against the wheel.

7.3 **REAGENTS**

7.3.1 Alkaline Mixture. 2 parts of $K_2CO_3 \cdot 1\frac{1}{2} H_2O$ plus 1 part $KHCO_3$. Mix thoroughly but do not powder. Dry or partially dry K_2CO_3 will cause the blood to clump and become warm. To keep salts moist, store in a closed container over saturated $K_2CO_3 . 1\frac{1}{2} H_2O$.

7.3.2 1N H_2SO_4. 1 part conc. H_2SO_4 in 35 parts of water.

7.3.3 Phenol Color Reagent. See Urea procedure 1.2.2.

7.3.4 Alkali-Hypochlorite Reagent. See Urea procedure 1.2.3.

7.3.5 Stock Ammonia Standard, 0.1 mg. N/ml. Dissolve 0.472 g. $(NH_4)_2SO_4$, A.R. grade, in distilled water in a 1 liter volumetric flash. Add a few drops of conc. H_2SO_4, then dilute to 1 liter with water. Stable indefinitely.

7.3.6 Working Ammonia Standard, 2 µg. N/ml. Dilute 2.0 ml. stock ammonia standard to 100 ml.

7.4 **PROCEDURE**

7.4.1 Set up a series of diffusion bottles (2 blanks, 2 standards and 2 for each unknown) containing 3.0 g. alkaline buffer mixture and 3 dry stainless steel rods. To each add 1.0 ml. H_2O.

7.4.2 Set up bottles in following order:

Blank. Add 1.0 ml. distilled water to bottle and immediately stopper.

Standard. Add 1.0 ml. working standard (2μg NH_3N) to bottle and immediately stopper.

Unknown. Add 1.0 ml. sample to bottle.

7.4.3 Mix on rotator for 1 min. Working with one bottle at a time, wet the ground portion of a receiving rod in IN H_2SO_4, remove the rubber stopper from the bottle while it is lying on its side, and quickly insert the receiving rod with stopper, taking care not to touch the neck of the bottle as the rod is inserted.

7.4.4 Rotate at 50 r.p.m. for 40 min.

7.4.5 Remove bottles from rotator and lay them down gently on their sides. Remove receiving rods one at a time carefully from the bottles, taking great care that the rod does not touch the neck of the bottle. Wash the acid off the receiving rod into a test tube with 1.0 ml. Phenol Color Reagent. Next, wash off the rod successively with 1.0 ml. alkali-hypochlorite reagent and 1.0 ml. H_2O.

7.4.6 Incubate in a water bath at 37°C for 20 min., or at 25°C for 40 min.

7.4.7 Add 5 ml. H_2O to all tubes mix.

7.4.8 Read Absorbance of blank (A_b), standard (A_s), and unknown (A_x) against water at 630 mu. If A_x is greater than 0.8, dilute the blank and unknown with water equally, read absorbance again, and make proper corrections in calculation.

7.5 **CALCULATION**

$$\mu g \text{ ammonia - N/100 ml.} = \frac{A_x - A_b}{A_s - A_b} \times 2 \times \frac{100}{I}$$

8. ON-SITE ANALYSIS OF pH, pCO_2, pO_2, AND HCO_3^- IN BLOOD AND PAROTID FLUID

8.1 REFERENCE

Henry, R.J., Clinical Chemistry: Principles and Techniques, pp. 435 - 467, 1964.

Model 160 Physiological Gas Analyzer Instruction Manual, Published by Spinco Division, Beckman Instruments, Inc., 1962.

8.2 APPARATUS

8.2.1 The Beckman Modular Cuvette.

8.2.2 Beckman Oxygen Macro Electrode.

8.2.3 Severinghaus - type pCO_2 Electrode.

8.2.4 Beckman Micro Blood pH Electrode.

8.2.5 The Model 160 Physiological Gas Analyzer.

8.2.6 Electrode Charging Material.

8.2.7 A 0-100 mv. range potentiometric strip chart recorder.

8.2.8 Oiled heparinized glass syringes for blood drawing.

8.2.9 Pre-weighed test tubes containing 0.5 ml. of mineral oil for anaerobic parotid fluid collection.

8.2.10 Compressed gases: 2% CO_2 in nitrogen and 5% CO_2 in nitrogen

8.3 **PROCEDURE**

8.3.1 Calibration of Electrodes. Proper settings in mm. Hg for the pCO_2 content of the calibrating gases were made in accordance with the following formula:

$$\text{Setting in mm.Hg} = \frac{\%\text{ of calibration } CO_2 \times (\text{barometric pressure} - \text{partial pressure of } H_2O \text{ vapor})}{100}$$

Partial pressure of water vapor @ 37°C is 47 mm. Hg. Both pCO_2 + pO_2 electrodes were operated in a saturated water vapor environment at 37°C.

Zero pO_2 was set against either one of the pCO_2 calibration gases. High pO_2 setting was made using room air (21% O_2 content) and substituting in the above equation.

Standard pH buffers corrected to 37°C were used to calibrate the pH electrode and to test span.

8.3.2 Blood. - Blood was drawn anaerobically from the anticubital vein into heparinized, oiled syringes. No tourniquet was used. After the proper amount of blood had been withdrawn, a few drops of mercury were drawn up into the syringe in order to effectively mix the sample. Immediately after mixing, the blood was charged into the cuvettes and values read out on the Gas Analyzer after an appropriate time lapse. Electrode drift was followed on the strip chart recorder and readings were taken until equilibrium had been reached. At least two repetitions of each function were made.

8.3.3 Parotid. - The proper volume of parotid fluid was collected anaerobically (under a layer of mineral oil) and aspirated into the cuvettes. Measurement procedure was identical to that of whole blood.

8.3.4 Following each series of measurements, the cuvette sample lines were flushed with distilled H_2O followed by isotonic saline prior to the introduction of a new specimen.

8.3.5 Calculation of HCO_3^- based on the determination of pH and pCO_2. The value of HCO_3^- concentration was calculated from the well-known Henderson-Hasselbalch equation by proper substitution:

$$pH = 6.10 + \log \frac{(HCO_3^-)}{0.0314\ pCO_2}$$

9. CHOLINESTERASE

9.1 REFERENCE

Michel, H.O., J. Lab. Clin. Med., 34, 1564 (1949).

Michel, H.O., Standard Methods of Clinical Chemistry, Vol. 3, ed. D. Seligson, Academic Press, N.Y., p. 93, 1961.

9.2 REAGENTS

9.2.1 Stock Buffer. - Place 44.73 g. KCl in a 250 ml. graduated beaker. Add hot distilled water to ca. 175 ml. and dissolve the salt. Wash 4.124 g. sodium barbital into a 200 ml. volumetric flask with about 100 ml. of the KCl solution and the sodium barbital dissolved by shaking. Dissolve 0.545 g. KH_2PO_4 in about 50 ml. of the KCl solution in a beaker. This solution and remaining KCl solution are transferred to the volumetric flask. Rinse beakers and add rinse to volumetric flask. Dilute to 200 ml. Store at room temperature in a polyethylene bottle.

9.2.2 Working Buffer Solution. - To 6.4 ml. stock buffer, add about 80 ml. H_2O and 8 ml. 0.01N HCl. Adjust pH to 8.00. Dilute to 100 ml. with water. When pH has dropped .05 pH unit, fresh buffer should be prepared.

9.2.3 Substrate. - To 100 mg. of acetylcholine chloride in a 15 ml. graduated tube, add water to 3.3 ml.

9.3 PROCEDURE

9.3.1 Standardize the pH meter with phospate buffer of about pH 7 and check operation with reference standards of lower pH. If electrodes have been used previously for alkaline solutions, soak in 0.1N

HCl for 10 min. prior to use.

9.3.2 Serum. - To 5 ml. distilled water in a 50 ml. beaker, add 0.10 ml. serum from a T.C. pipet, followed by 5 ml. working buffer solution. Swirl to mix and let stand at 25°C for about 10 min. Parotid Fluid. - To 1.0 ml. parotid fluid in a 5 ml. beaker, add 1.0 ml. working buffer solution. Mix and let stand 10 min. at 25°C.

9.3.3 Take the pH of sample, rinsing the electrodes with distilled water and wiping dry with tissue prior to each measurement. This is pH_1. Add 1 ml. substrate to serum sample or 0.2 ml. substrate to parotid fluid sample. Mix and note time. Times of addition of substrate to a series of samples should be staggered, e.g., at 1 or 2 min. intervals.

9.3.4 After 55 min., restandardize pH meter. Take pH's of samples exactly 1 hour after addition of substrate. This is pH_2.

9.4 CALCULATION

SERUM cholinesterase units $= \Delta$ pH/hr.

$$= pH_1 - pH_2 - b$$

PAROTID FLUID cholinesterase units $= \frac{\Delta \text{ pH/hr.}}{50}$

The value for b is obtained from the following table: (correction for the drop in pH resulting from non-enzymatic hydrolysis during the period of incubation)

pH_2	b	pH_2	b	pH_2	b
7.9	0.09	7.5	0.04	7.1	0.02
7.8	0.07	7.4	0.03	7.0	0.01
7.7	0.06	7.3	0.02	6.8	0.01
7.6	0.05	7.2	0.02	6.6	0.01

MATERIAL	Sensitivity*	Recovery**	Precision***
Ammonia-N	10^{-2} ppm	100%	± 8%
Calcium	8×10^{-2} ppm	95%	± 3%
Protein	10 ppm		± 3%
Urea-N	1 ppm	95%	± 10%
Glucose	1 ppm	92%	± 2%
Bicarbonate			± 2%
pO_2	1 mm of Hg	100%	± 2%
pCO_2	1 mm of Hg	100%	± 1%

* The smallest amount of material which will produce a change in the reading.

** The percent of material recovered when added to parotid fluid

*** The random error, the variation of results obtained by a method when the same sample is run repeatedly